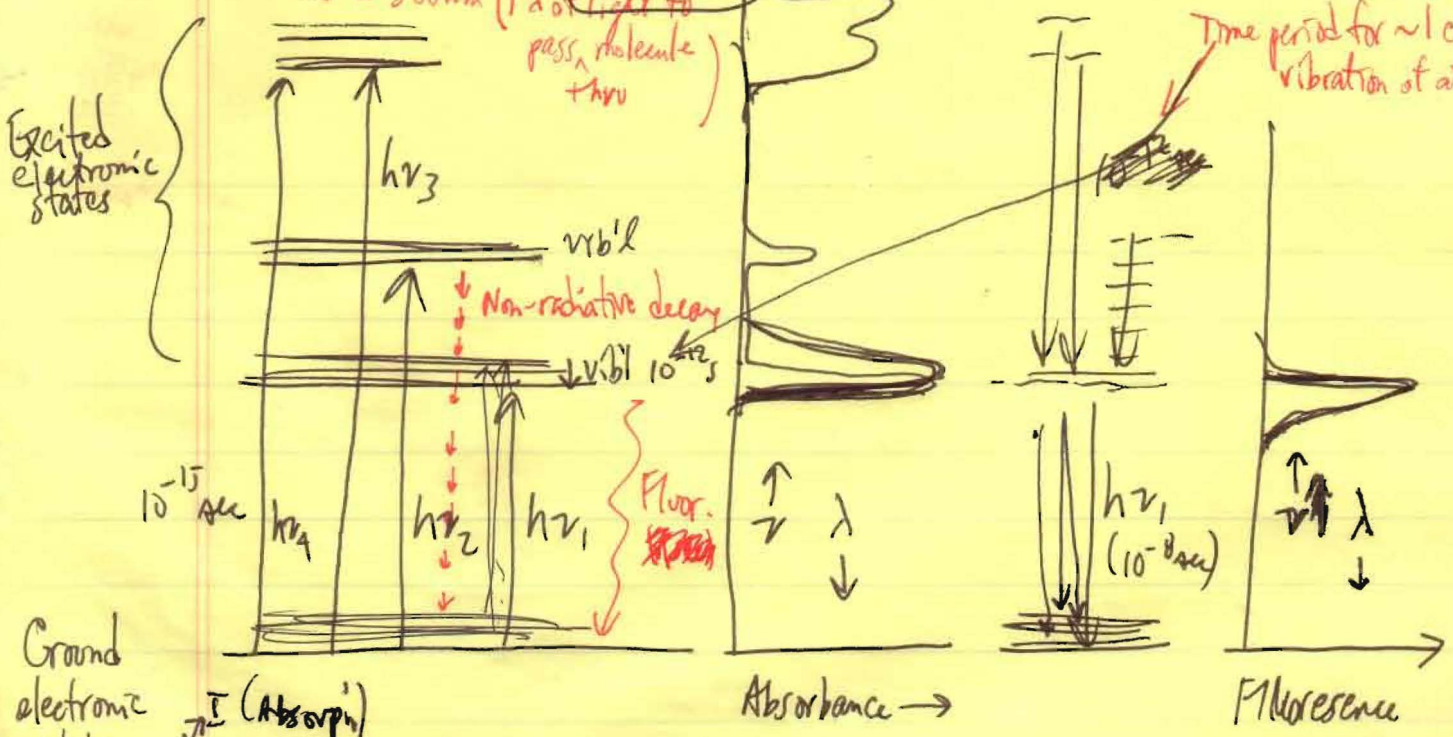
$c = 3\times10^{8}$ m/sec $\times 10^{-8}$ sec $= 3\times10^{-1}$ m
$= \frac{3\times10^{-7}\text{ m}\times10^{9}\text{ nm/m}}{} = 300$ nm (1 λ of light to pass a molecule + hν)

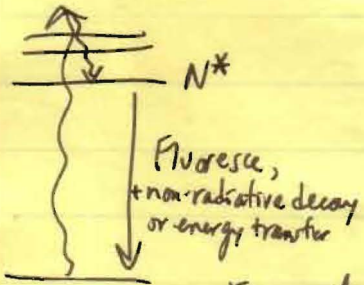
Time period for ~1 cycle of vibration of atoms



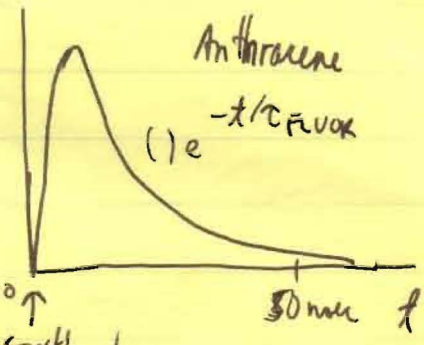
Note: Fluorescence ν is lower than absorbance ν.

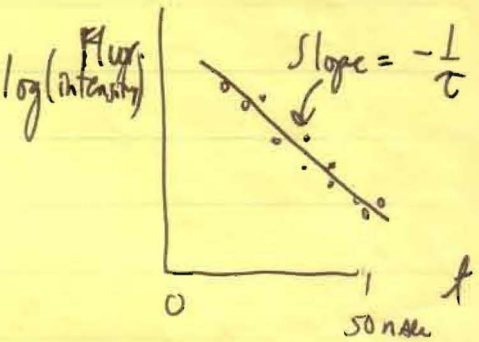
Exc'n spectrum (Scan ν_{IN} Obs all Fluor ν); Emission spectrum (Fix ν_{IN}, Scan ν_{out})

Parameters: ν, τ lifetime) Quantum yield (quenching), donor-acceptor energy transfer, polariz'n ↕ vs ↔ (INTENSITY)



$$\frac{dN^*}{dt} = -kt = -\frac{t}{\tau_{\text{FLIFETIME}}} \qquad 4 < \tau < 30 \text{ nsec}$$





Quantum yield = $\frac{\#\text{ photons emitted as fluorescence}}{\#\text{ photons absorbed}}$ (incident)

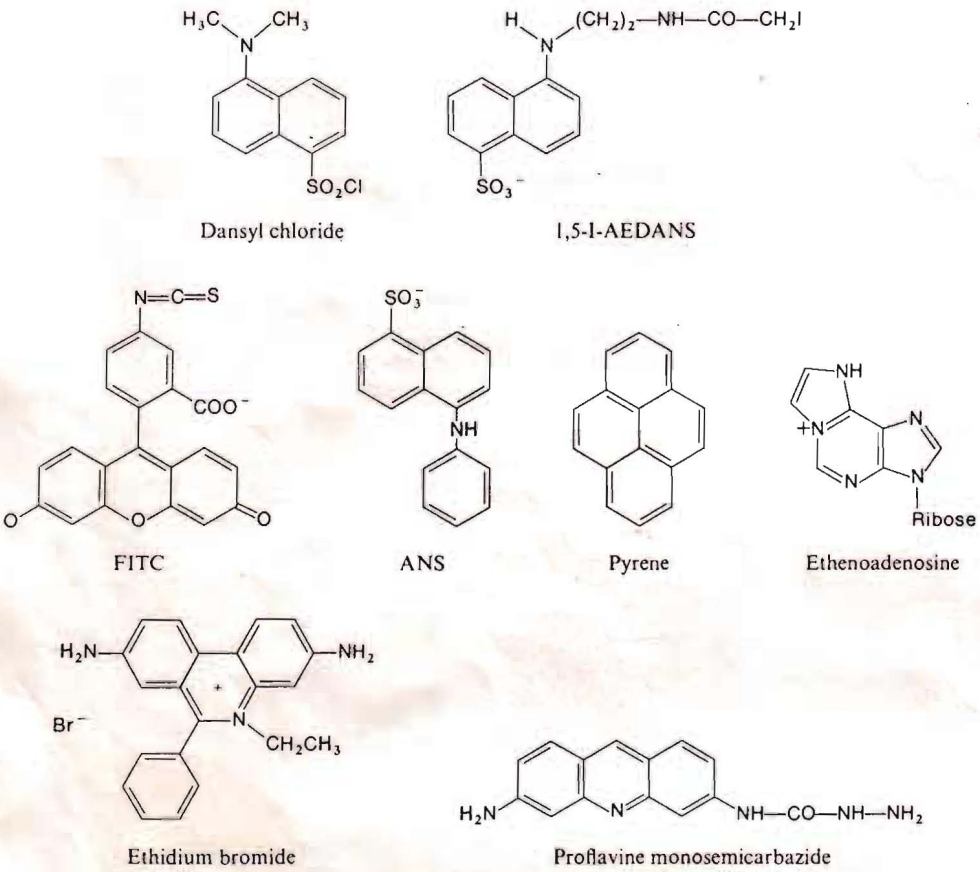


Figure 8-16

Structures of fluorescent probes listed in Table 8-3.

In contrast, during the 10^{-9} to 10^{-8} sec that a singlet remains excited, all kinds of processes can occur, including protonation or deprotonation reactions, solvent-cage relaxation, local conformational changes, and any processes coupled to translational or rotational motion.

A number of fluorescent molecules have a very convenient property: in aqueous solution their fluorescence is very strongly quenched, but in a nonpolar or a rigid environment a striking enhancement is observed. This enhancement can easily be by more than a factor of 20. If the probe can bind to a rigid or nonpolar site on a protein or nucleic acid, the fluorescence spectrum will be dominated by the bound species. Figure 8-17 shows a typical example.

For proteins, the dye 8-anilinonaphthalene sulfonate (ANS) is the most frequently used environmental probe, although several other common ones exist. Ethidium is

Table 8-3
Typical fluorescent probes

Probe[a]	Uses	Absorption		Emission[§]			Sensitivity
		λ_{max} (nm)	$\varepsilon_{max} \times 10^{-3}$	λ_{max} (nm)	ϕ_F	τ_F (nsec)	$\varepsilon_{max}\phi_F \times 10^{-2}$
Dansyl chloride	Covalent attachment to protein: Lys, Cys	330	3.4	510	0.1	13	3.4
I,5-I-AEDANS	Covalent attachment to protein: Lys, Cys	360	6.8	480	0.5	15	34
Fluorescein isothiocyanate (FITC)	Covalent attachment to protein: Lys	495	42	516	0.3	4	116
8-Anilino-1-naphthalene sulfonate (ANS)	Noncovalent binding to proteins	374	6.8	454	0.98	16	67
Pyrene, and various derivatives	Polarization studies on large systems	342	40	383	0.25	100	100
Ethenoadenosine, and various derivatives	Analogs of nucleotides bind to proteins, incorporate into nucleic acids	300	2.6	410	0.40	26	10
Ethidium bromide	Noncovalent binding to nucleic acids	515	3.8	600	~1	26.5	38
Proflavine monosemicarbazide	Covalent attachment to RNA 3′-ends	445	15	516	0.02	—	30

[§] Values shown for ϕ_F and τ_F are near the maximum typically observed in biological samples at ambient temperature. Other (considerably smaller) values often are found.
[a] Structures of these probes are shown in Figure 8-16.

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Intensity

Table 10.5 Fluorescence quantum yields and radiative lifetimes

Compound	Medium	τ, ns	ϕ	Reference*
Fluorescein	0.1 *M* NaOH	4.62	0.93	a
Quinine sulfate	0.5 *M* H_2SO_4	19.4	0.54	a
9-Aminoacridine	Ethanol	15.15	0.99	a
Phenylalanine	H_2O	6.4	0.004	b
Tyrosine	H_2O	3.2	0.14	b
Tryptophan	H_2O	3.0	0.13	b
Cytidine	H_2O, pH 7	—	0.03	c
Adenylic acid (AMP)	H_2O, pH 1	—	0.004	c
Etheno-AMP	H_2O, pH 6.8	23.8	1.00	d
Chlorophyll a	Diethyl ether	5.0	0.32	e
Chlorophyll b	Diethyl ether	—	0.12	e
Chloroplasts	H_2O	0.35–1.9	0.03–0.08	f
Riboflavin	H_2O, pH 7	4.2	0.26	g
DANSYL sulfonamide†	H_2O	3.9	0.55	h
DANSYL sulfonamide + carbonic anhydrase	H_2O	22.1	0.84	h
DANSYL sulfonamide + bovine serum albumin	H_2O	22.0	0.64	h

* (a) W. R. Ware and B. A. Baldwin, *J. Chem. Phys. 40*, 1703 (1964); (b) R. F. Chen, *Anal. Letters 1*, 35 (1967); (c) S. Udenfriend, *Fluorescence Assay in Biology and Medicine*, Vol. II, Academic Press, New York, 1969; (d) R. D. Spencer et al., *Eur. J. Biochem. 45*, 425 (1974); (e) G. Weber and F. W. J. Teale, *Trans. Faraday Soc. 53*, 646 (1957); (f) A. Müller, R. Lumry, and M. S. Walker, *Photochem. Photobiol. 9*, 113 (1969); (g) R. F. Chen, G. G. Vurek, and N. Alexander, *Science 156*, 949 (1967); (h) R. F. Chen and J. C. Karnohan, *J. Biol. Chem. 242*, 5813 (1967).

† DANSYL sulfonamide is 1-dimethylaminonaphthalene-5-sulfonamide.

(The molecule azulene is an example of one of the rare exceptions; azulene fluorescence comes predominantly from the second excited singlet state.) The reason that only the lowest state normally emits radiation is that the processes of internal conversion of the higher states (thermal deactivation from higher electronic states to the lowest excited state) are exceedingly rapid. This is illustrated for bacteriochlorophyll in Fig. 10.16, where the absorption and fluorescence spectra are plotted in the vertical direction (turned 90° from the usual orientation) to correspond to the energy-level diagram. Internal conversion from the lowest excited state to the ground state also occurs. It is one of the important sources of thermal deactivation, $k_t[M^*]$, that compete with fluorescence. The rate is often slower for this step, however, partly because of the greater energy separation between the ground state and the first excited electronic state compared with the energy differences among the excited states.

Fluorescence Quenching

A decrease in fluorescence intensity or quantum yield occurs by a variety of mechanisms. These include collisional processes with specific quenching

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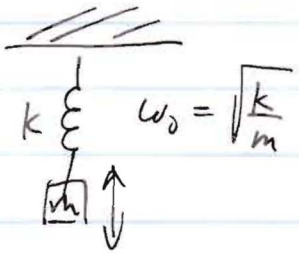
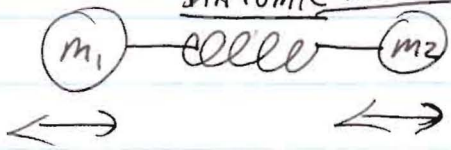
VIBRATING MOLECULES

START W. WT ON SPRING AGAIN, WITH TWO NEW WRINKLES



DIATOMIC MOLECULE



$$\omega_0 = 2\pi\nu_0 = \sqrt{\frac{K}{\mu}}$$

$\mu = \frac{m_1 m_2}{m_1 + m_2}$ = "REDUCED" MASS $\left(\frac{1}{\mu} = \frac{1}{m_1} + \frac{1}{m_2}\right)$

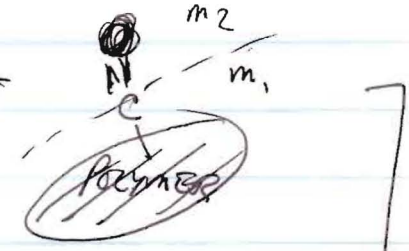
Suppose $m_1 \gg m_2 \Rightarrow \mu \cong \frac{m_1 m_2}{m_1} \cong m_2$

$m_1 = m_2$

$\mu = \frac{m^2}{2m} = \frac{1}{2}m$

MAKES VIB'L SPECTRA USEFUL DIAGNOSTICALLY

~~$m_1 = 10 m_2 \Rightarrow \mu = 0.91 m_2$~~



"GROUP VIB'L FREQ"
FOR SMALL GROUP BOUND TO MASSIVE MOLECULE

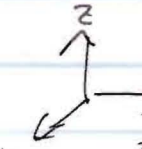
Q. HOW MANY VIBRATIONS ARE THERE?

A. "NORMAL MODES" (COORD'S.) : WAYS IN WHICH ALL ATOMS IN A MOLECULE MOVE SYNCHRONOUSLY TOGETHER

FOR N-ATOM MOLECULE, # POSSIBLE MOTIONS = $3N$ (i.e., x_i, y_i, z_i FOR N ATOMS)

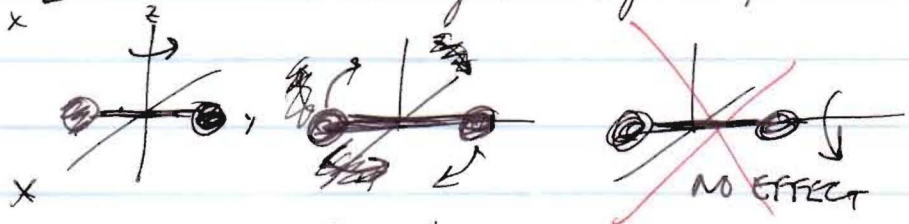
$3N-5$ (LINEAR) OR $3N-6$ (NON-LIN) VIB'NS

BUT 3 TRANSLATIONS



All move together along x or y or z

2 OR 3 ROTATIONS



(DIATOMIC) OR LINEAR

ALL OTHER TRIATOMIC + NONLINEAR

DIATOMIC $3 \cdot 2 - 5 = 1$ VIB'N, 2 ROT'NS, 3 TRANSL'NS

SEE EXAMPLES: O=C=O (LINEAR)

NON-LINEAR

PATTERN CAN BE CALC'D, MASSES ARE KNOWN, FIT SPECTRAL PEAK FREQ'S TO FORCE CONST'S FOR EACH "STRETCH" OR "BEND"

$3 \cdot 3 - 5 = 4$ VIB'NS, 2 ROT'NS

$3 \cdot 3 - 6 = 3$ VIB'NS, 3 ROT'NS

Find center of mass

In general, the kinematic description of a molecule composed of N atoms requires $3N$ variables: e.g., 3 Cartesian position coordinates for each of N atoms. However, as we begin to see from the preceding example, the motions of the atoms are more conveniently represented in terms of "normal" coordinates. For a diatomic (or any other) molecule, three of the normal coordinates correspond to *translations* along x- or y- or z-axes (e.g., q_2, q_4, and q_6 in Eqs. 9.2 and Figure 9.2). For a diatomic molecule, one of the normal modes (q_1 in Figure 9.1) is a *vibration*. The remaining two normal modes (q_3 and q_5 in Eqs. 9.2 and Figure 9.2) of a diatomic molecule are *rotations*. Just as all of the atoms move together in any one of the three *translations*, the atoms all *rotate* or *vibrate* at a common frequency along a given rotational or vibrational normal coordinate.

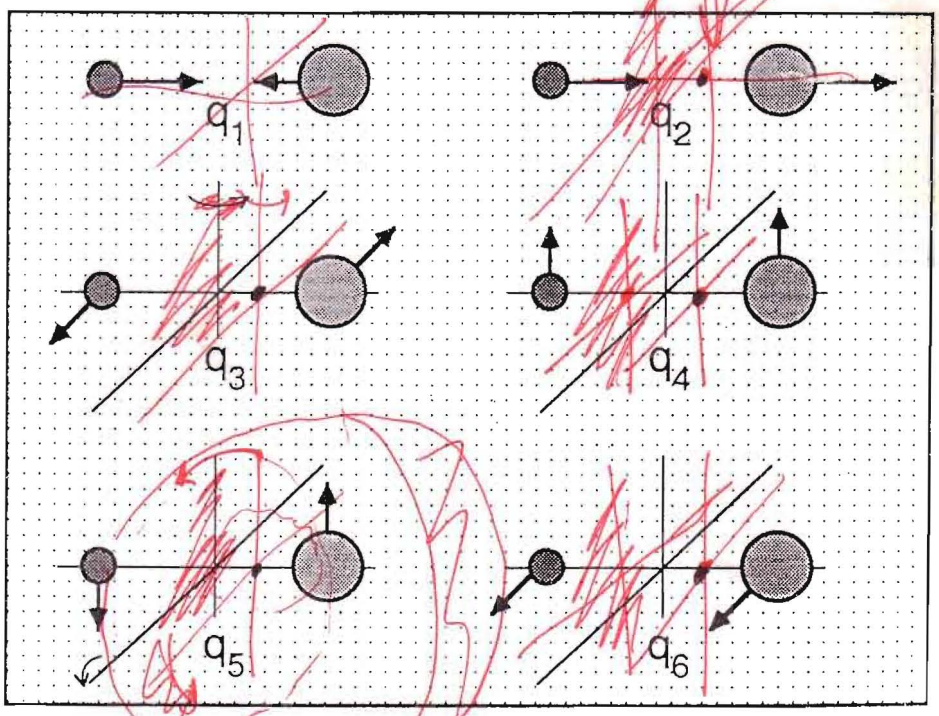
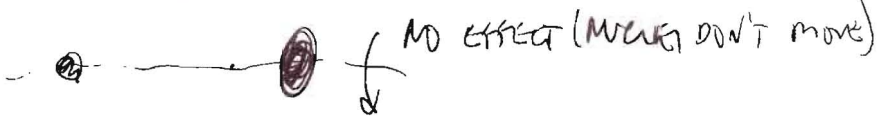


Figure 9.2 Motions along each of the six "normal" coordinates for a diatomic molecule. q_1 is the vibrational coordinate; q_2, q_4, and q_6 represent translations; and q_3 and q_5 are rotations.



For the harmonic potential (i.e., quadratic distance-dependence) of Eq. 9.14a, we already know that the solution will be a sinusoidal oscillation. With that assumption, the values of λ_i in Eq. 9.14a may be obtained in a systematic classical mechanical "FG-matrix" method, to yield the various "normal mode" vibrational frequencies (see Further Reading). However, quantum mechanics is needed to determine the relative *amplitudes* of the various normal mode vibrations, and the *selection rules* (namely, which vibrations will be observed in a given experiment).

Some representative results are shown in Figure 9.3 for a linear triatomic molecule (CO_2, with 2 rotational and $3N - 5 = 4$ vibrational modes) and a non-linear triatomic molecule (SO_2, with 3 rotational and $3N - 6 = 3$ vibrational modes). Vibrations with displacements *along* the axes of chemical bonds are known as "stretches", and vibrations with displacements in other directions are variously known as "bending", "twisting", "rocking", etc.

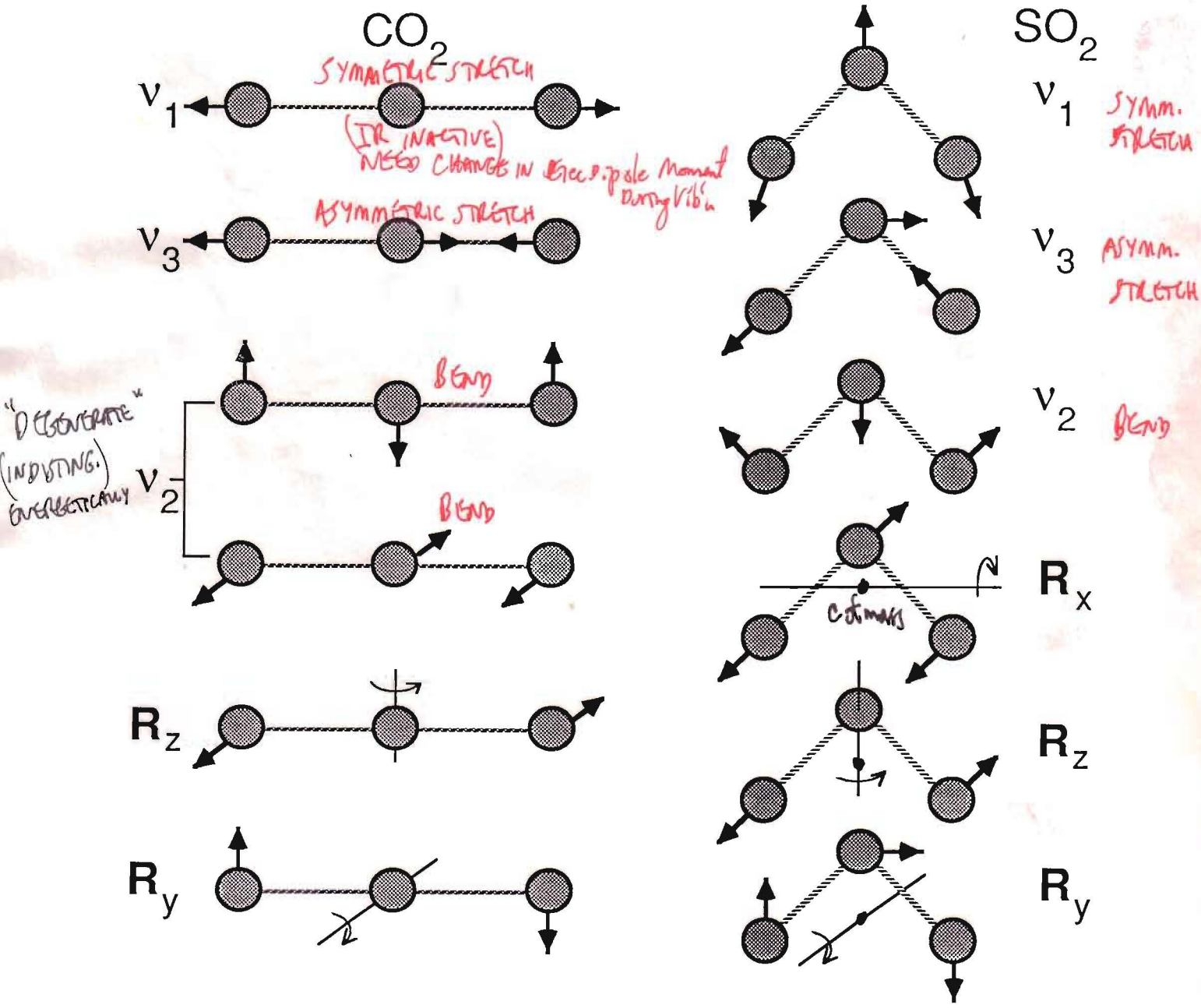


Figure 9.3 Normal modes of vibration and rotation of linear (CO_2) and non-linear (SO_2) triatomic molecules. The three translational modes are not shown.