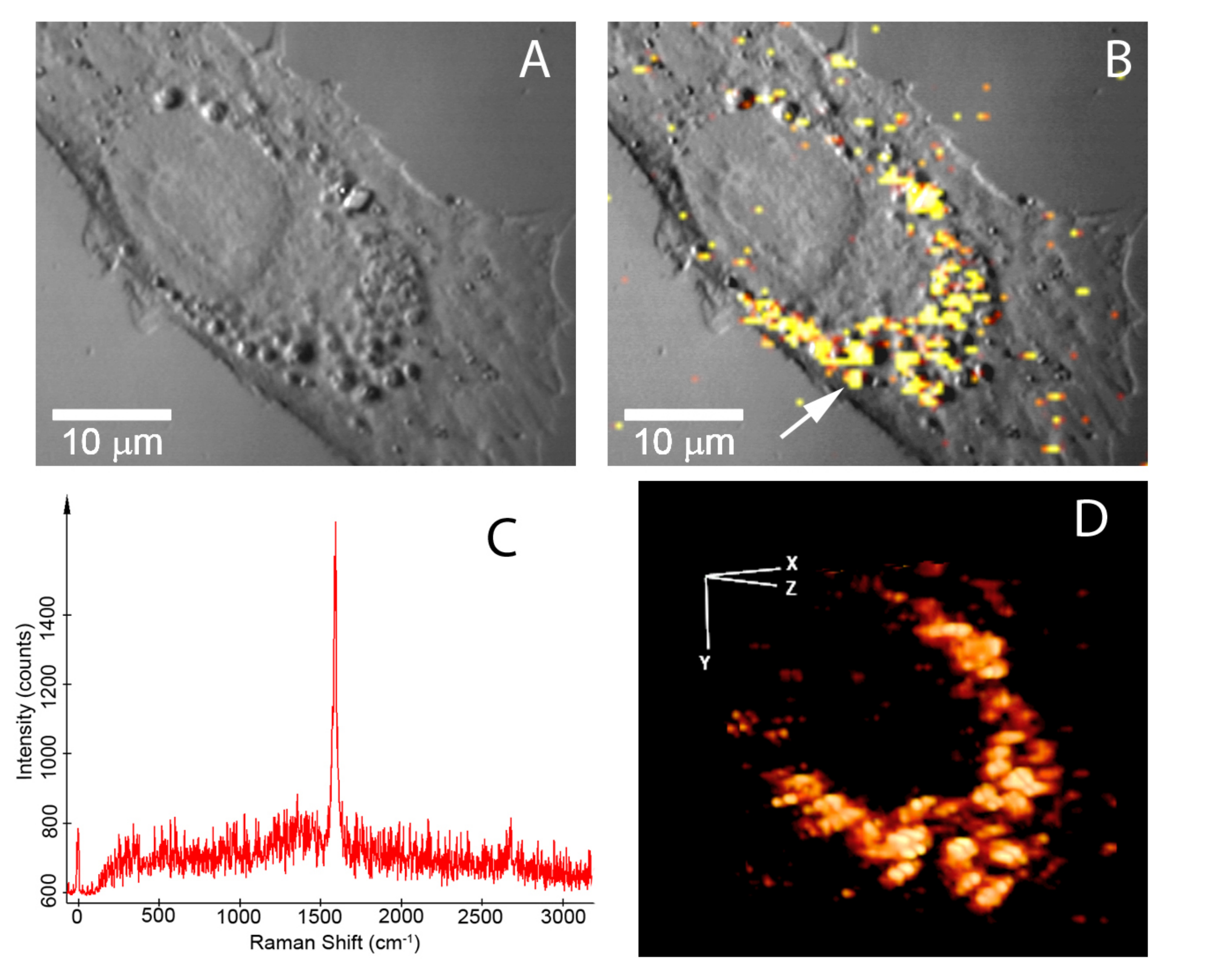
Advantages of Raman: can use any photon wavelength you want. If you use visible light:

1. can avoid absorption of light by H_2O, CO_2, etc.

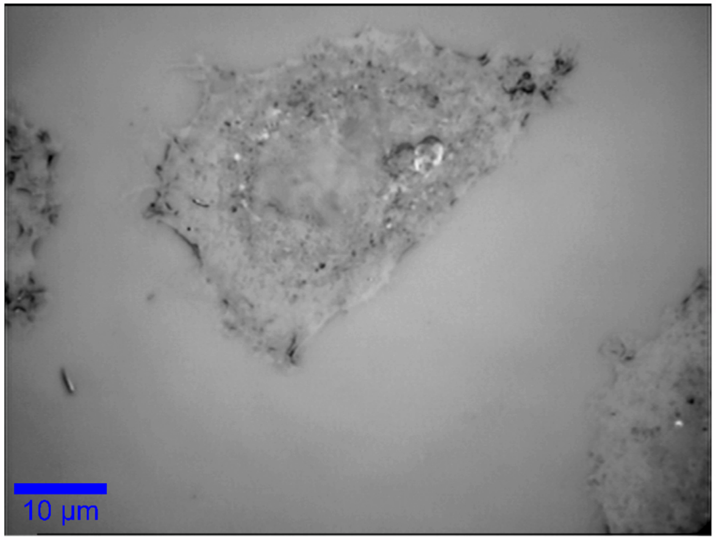
2. can use optical microscopy to focus with a spatial resolution better than 0.01 mm. This is called “Raman microscope” or “microRaman”.

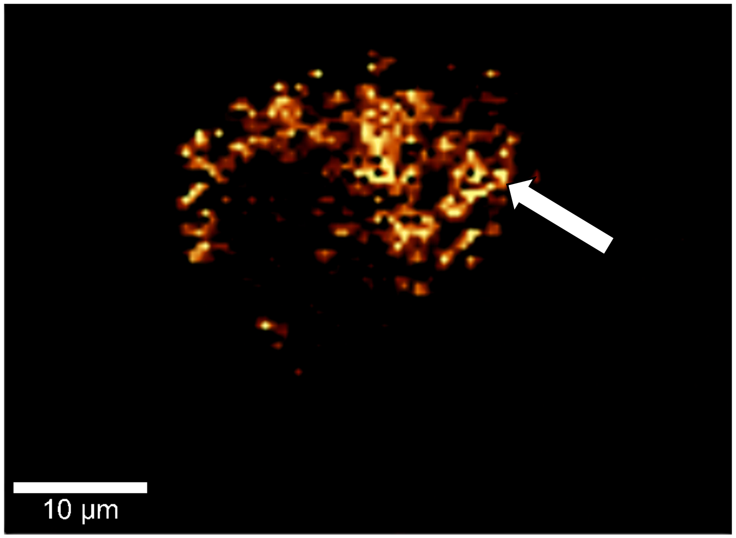
Confocal Raman Microscopy

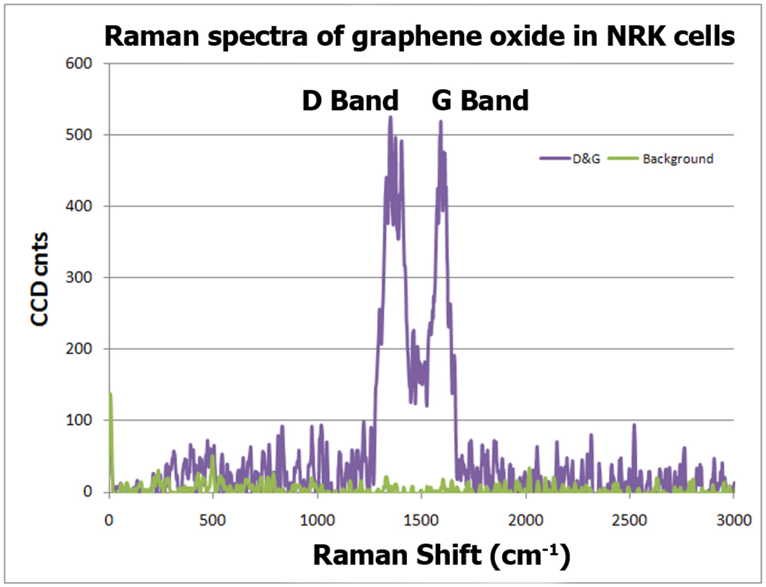
Single-walled Carbon Nanotubes Inside Mammalian Cells

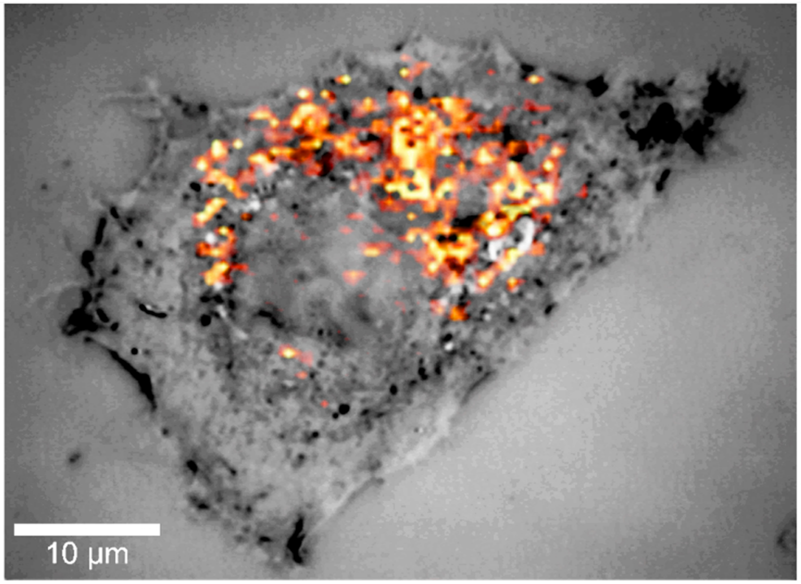


The Interaction of Graphene Oxide with Mammalian Cells









CARS: coherent anti-Stokes Raman spectroscopy

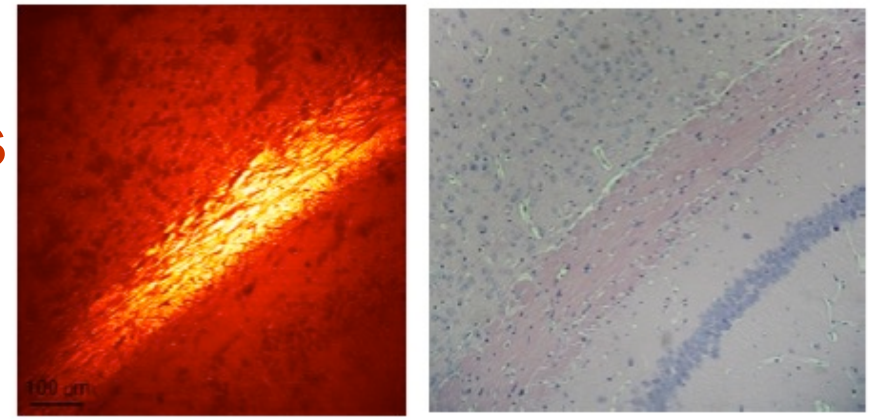


Figure 5. Epi-CARS microscopy image and histology (H&E stained) image of the similar region of the brain shown in Figure 1.

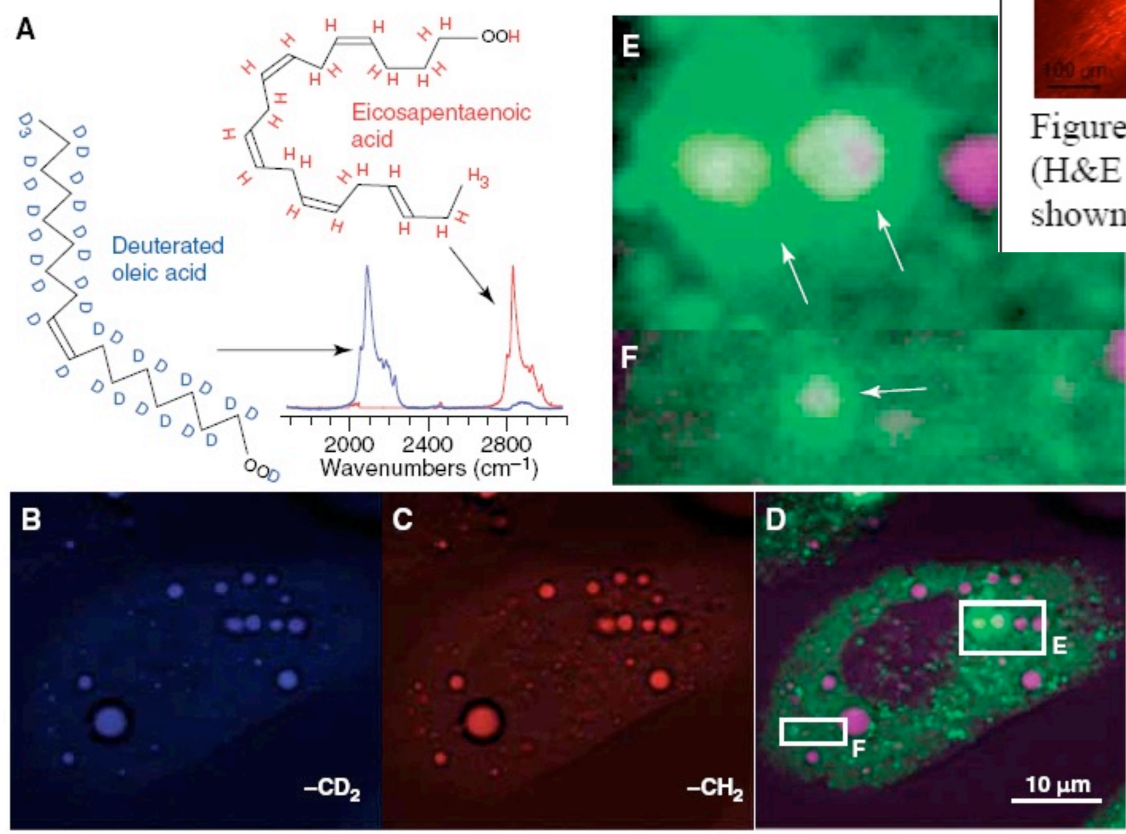


Fig. 3. Effect of fish oil on lipid metabolism studied by metabolic imaging with CARS microscopy. (**A**) Raman spectra of EPA and deuterated OA. (**B** to **F**) Live liver cells were treated with 0.4 mM EPA and 0.2 mM deuterated OA for 7.5 hours and labeled with monodansylcadaverine, a dye for staining degradative organelles. (B) CARS image tuned to $-CD_2$ (blue, deuterated OA). (C) CARS image tuned to $-CH_2$ (red, EPA). (D) Composite image of well-mixed $-CO_2$ and $-CH_2$ (purple) and two-photon fluorescence from monodansylcadaverine (green). [(E) and (F)] Zoomed-in regions in the cell where triglycerides rich in $-CH_2$ and $-CD_2$ are colocalized within degradative compartments (stained by monodansylcadaverine and indicated by arrows).

Use two laser beams of frequency ω_1 and ω_2

The beams overlap, creating an electric field with frequency component $\omega_1 - \omega_2$ (among others)

If this frequency difference is equal to a vibrational frequency in the sample, get dramatically enhanced signal (resonance or coherence)

Can be used for non-invasive functional biological imaging

Infrared spectroscopy on larger molecules

Infrared radiation from 300 cm^{-1} to 3000 cm^{-1} (which we sense as warmth) is absorbed primarily by molecular vibrations. The vibrations determine the peak positions, and rotational motion adds fine structure to the peaks. But this fine structure is lost for larger molecules or even for small molecules in the liquid phase.

For HCl the spectrum was easy to understand. For polyatomic molecules things are more complicated. Each fundamental type of vibration of a polyatomic molecule is called a normal mode.

Normal Modes (from Engel/Reid "Physical Chemistry")

1. During a vibrational period, the center of mass remains fixed and all the atoms undergo in-phase periodic motion about their equilibrium positions.
2. All atoms reach their minimum and maximum amplitudes at the same time.
3. These collective motions are called normal modes and the frequencies are called normal mode frequencies.
4. The frequencies measured in vibrational spectroscopy are the normal mode frequencies.
5. All normal modes are independent in the harmonic approximation, meaning that excitation of one normal mode does not result in any energy transfer into any other mode.
6. Any seemingly random motion of the atoms in a molecule can be expressed as a linear combination of the normal modes of that molecule.

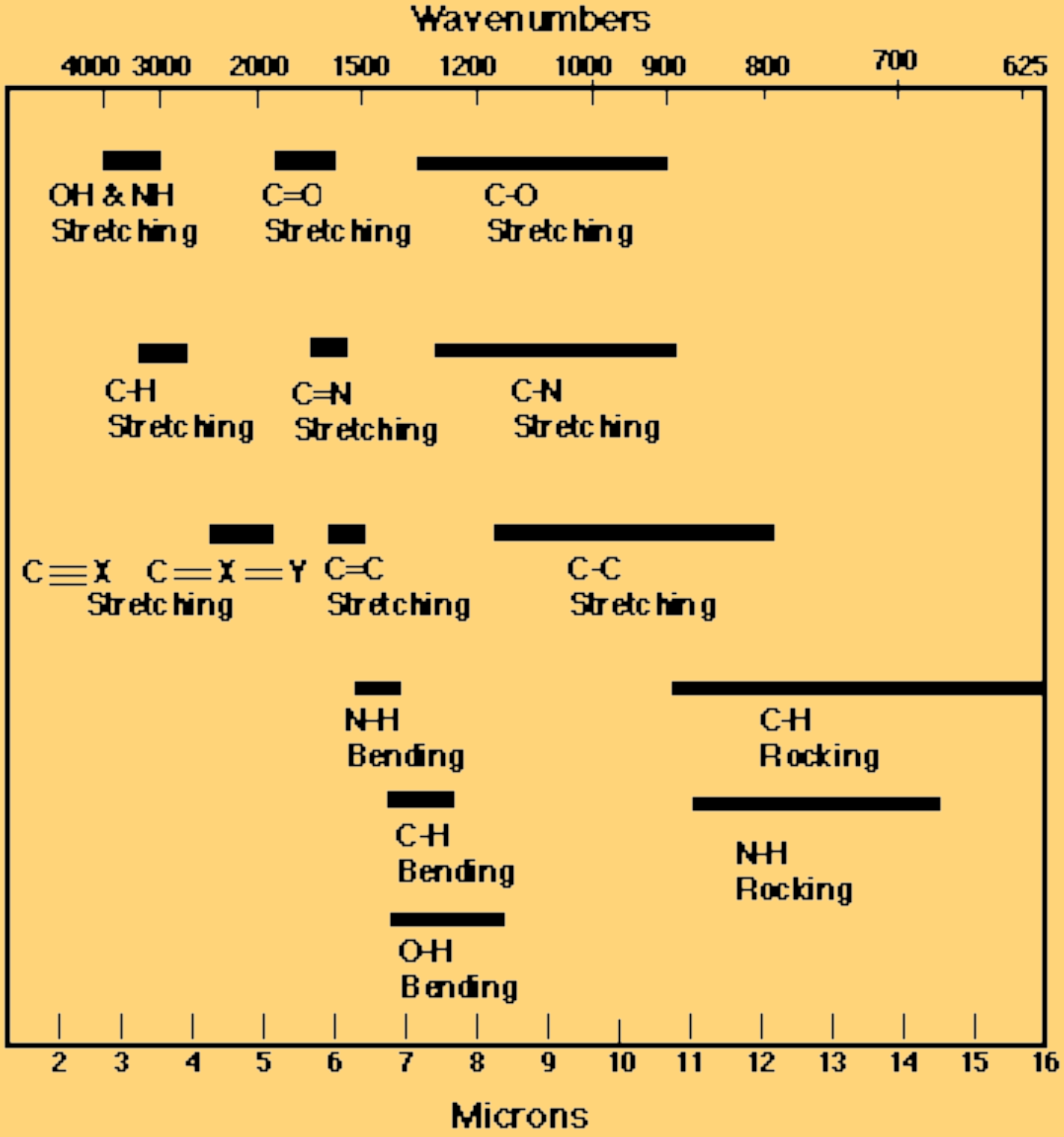
BASIS

How do we deal with the complicated spectra of large molecules?

(which depend on the environment – in water, in gas phase, etc.)

1. Could try to calculate every possible vibrational mode using quantum mechanics. Not practical.
2. Often, we don't care about every possible mode – are certain functional groups present in the molecule? It turns out that some normal modes involve the motion of just a few of the atoms. Most useful for qualitative analysis of organic compounds, and for monitoring the progress of organic reactions.
3. Sometimes, we don't care what the modes are: we just want to compare the spectrum against a reference library of known compounds. This is a common procedure in environmental and forensic analysis.

Chart of Characteristic Vibrations



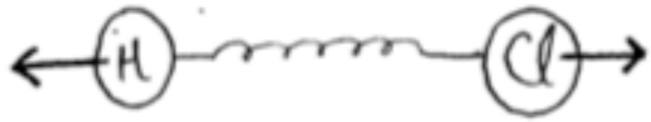
A linear molecule like CO_2 containing N atoms has 3N-5 normal modes of vibration. So CO_2 has 4 normal modes and HCl has 1 normal mode

A non linear molecule like CH_2Cl_2 containing N atoms has 3N-6 fundamental modes of vibration, so methylene dichloride has 9 normal modes.

Normal modes can be stretching or bending motions

- stretching (symmetric and antisymmetric)
- bending (scissoring, rocking, wagging, twisting)

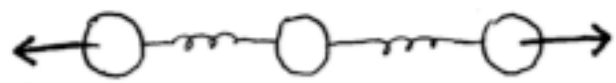
HCl linear : 3(2) − 5 = 1 normal mode



(active)

CO_2 linear : 3(3) − 5 = 4 normal modes

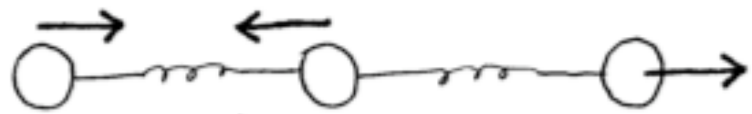
mode #1 :



(inactive)

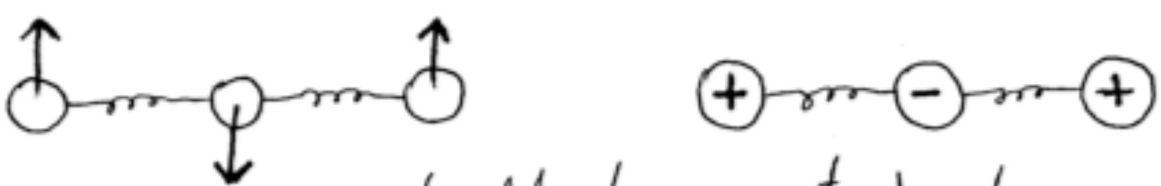
symmetric stretch $\nu = 1388.3 \text{ cm}^{-1}$

mode #2 :



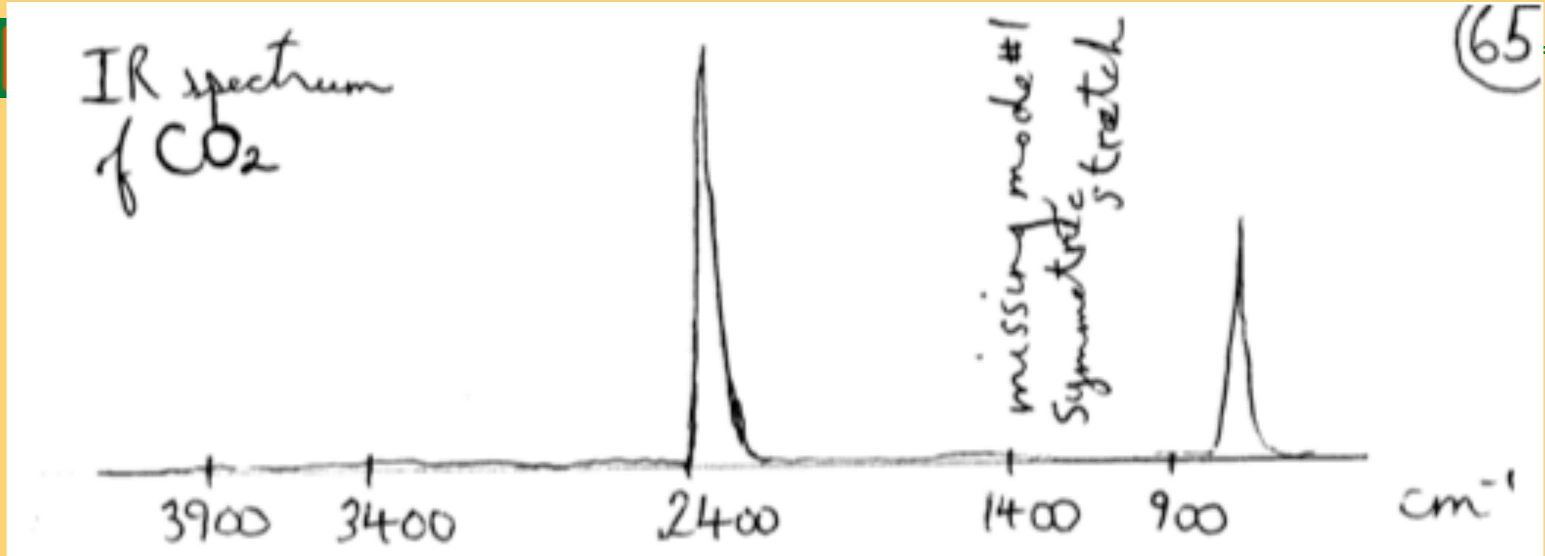
antisymmetric stretch $\nu = 2349.3 \text{ cm}^{-1}$

modes #3 and 4



doubly degenerate bending mode

$\nu = 667.3 \text{ cm}^{-1}$



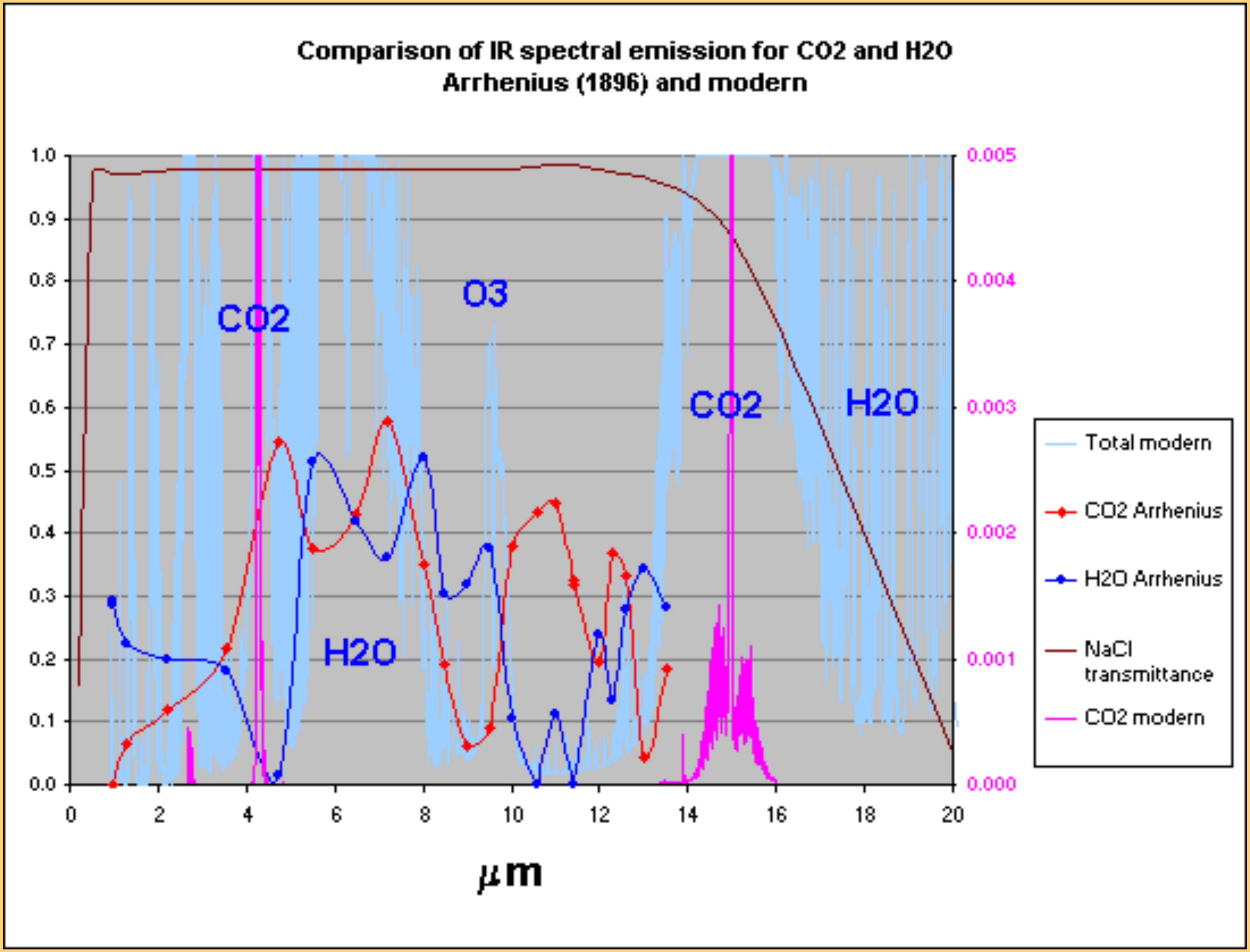
CH_2Cl_2 non-linear $5 \cdot 3 - 6 = 9$ normal modes

see animations of these modes + spectrum at

http://cat.middlebury.edu/~chem/chemistry/ch2cl2/vib.html#2

(need a special plugin)

animation



Comparison of IR spectral emission for CO2 and H2O
Arrhenius (1896) and modern
CO2
O3
H2O
CO2
H2O
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0.005
0.004
0.003
0.002
0.001
0.000
0
2
4
6
8
10
12
14
16
18
20
μm
Total modern
CO2 Arrhenius
H2O Arrhenius
NaCl transmittance
CO2 modern

UTD

Why is the spectrum so complicated? In addition to the peaks centered on the fundamental (normal mode) frequencies, other peaks appear in the spectra of polyatomic molecules.

Some of these can be because of violations of the $\Delta n = \pm 1$ selection rule due to anharmonicities.

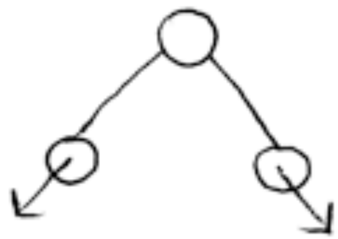
But in polyatomic molecules, combination bands also appear.

For example, normal mode 1 can go from $n_1 = 0 \rightarrow 1$ and simultaneously normal mode 2 can go from $n_2 = 0 \rightarrow 1$. This will give an absorption peak centered at $\nu_1 + \nu_2$.

In particular, water shows strong combination bands.

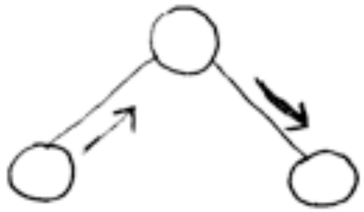
H_2O non-linear $3 \cdot 3 - 6 = 3$ normal modes

mode #1



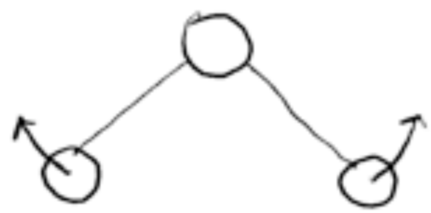
symmetric stretch
$\nu = 3657.05\ cm^{-1}$

mode #2



asymmetric stretch
$\nu = 3755.93\ cm^{-1}$

mode #3

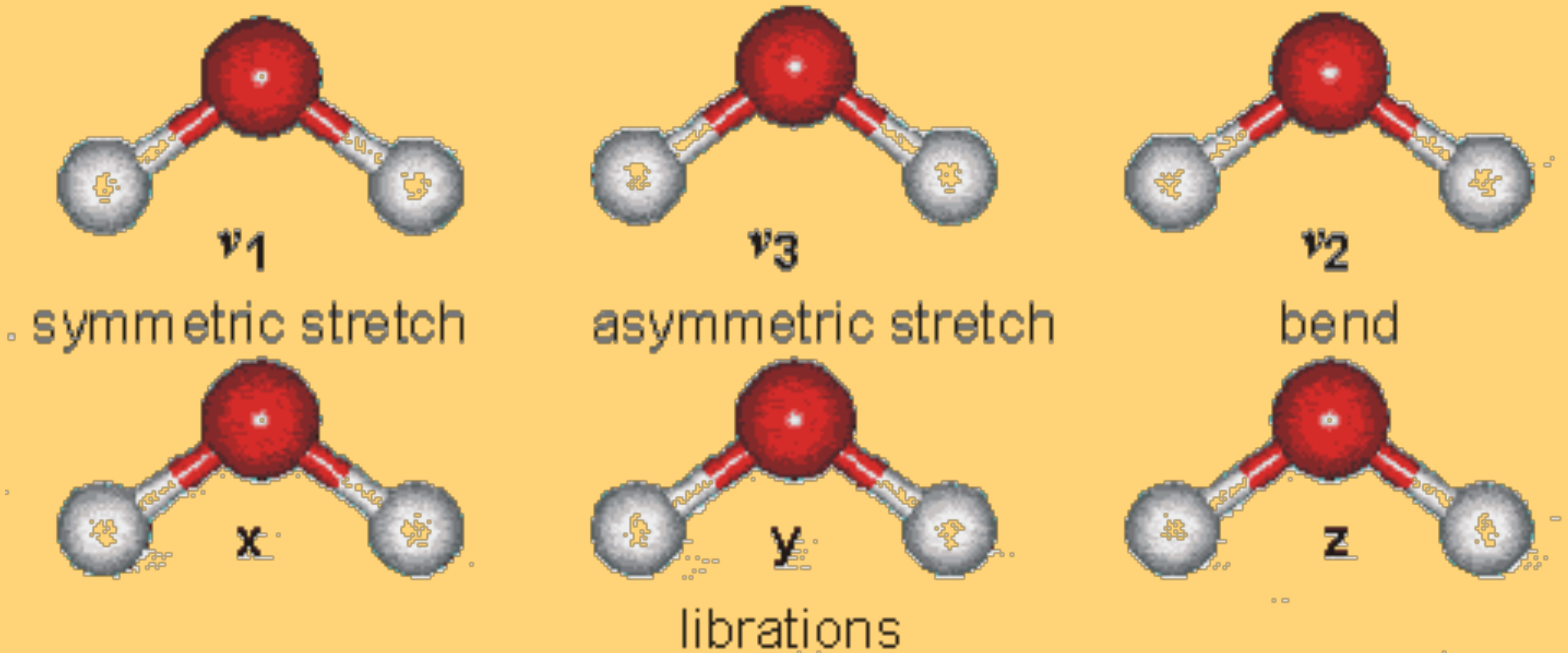


bend
$\nu = 1594.75\ cm^{-1}$

For animations see http://www.lsbu.ac.uk/water/vibrat.html

animation

Normal modes for water vapor and librations for liquid water



This is for an isolated molecule (gas phase). What about liquid water? have hydrogen bonding

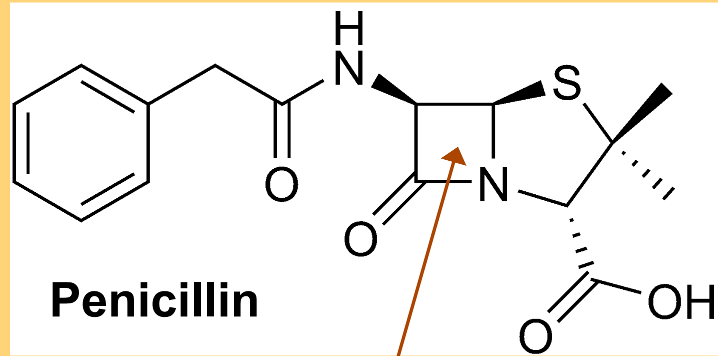
Variations in the environment around each liquid water molecule gives rise to significant broadening and shifting of the peaks.

In addition, combinations of vibrations with librations give many additional peaks. Librations are restricted rotations ie rocking motions

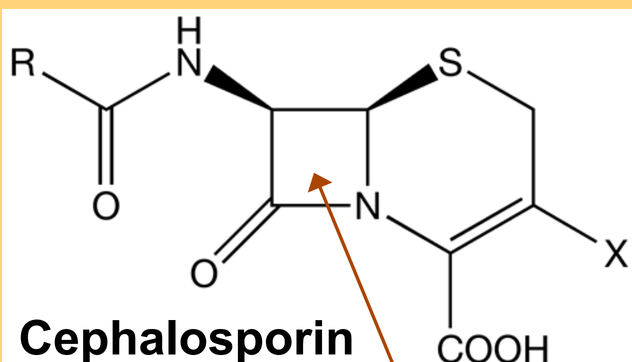
See web site given above.

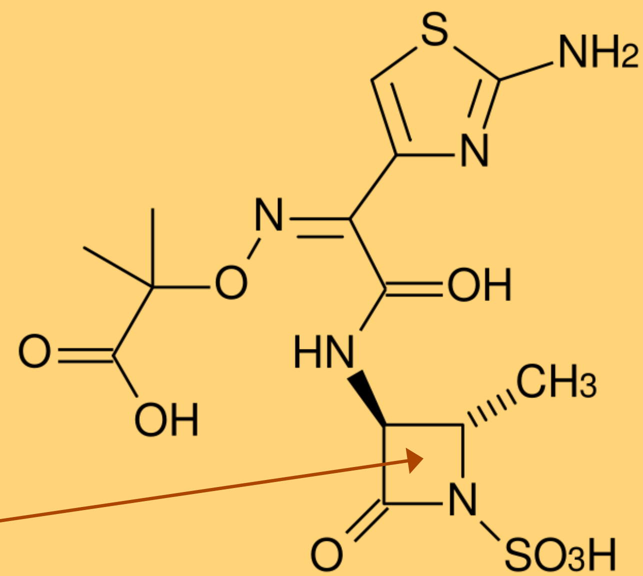
β-lactam antibiotics

β-Lactam antibiotics, such as penicillins and cephalosporins, inhibit biosynthesis of bacterial cell walls by acylating and thereby inactivating transpeptidases and carboxypeptidases.



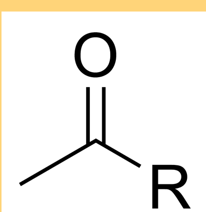
β-Lactam ring



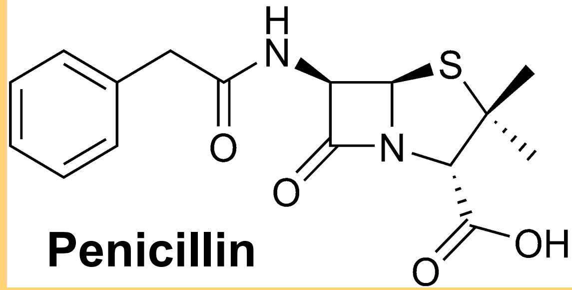


β-Lactam ring

Aztreonam



β-lactam antibiotics



Penicillin

Because the antibacterial activity of an antibiotic depends on the acylation of those enzymes by the **β**-lactam ring of the antibiotic, the chemical reactivity that represents the acylating ability of the **β**-lactam ring is an important factor affecting the antibacterial activity.

Thus, much interest has been attached to investigation of the structure-reactivity relationship of cephalosporins and penicillins as the first stage in the prediction of antibacterial activity. A number of parameters have been proposed as indicators of the **β**-lactam reactivity, for example, the IR carbonyl stretching frequency (**β**-lactam $\nu_{C=O}$).

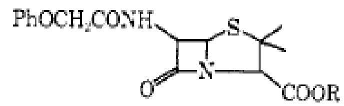
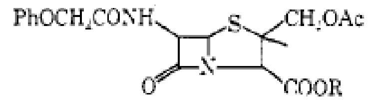
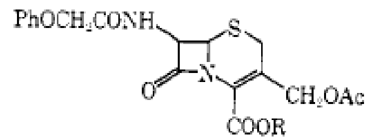
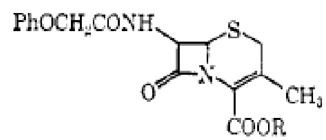
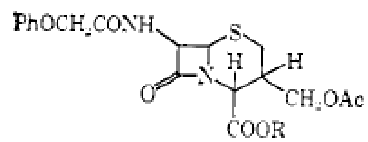
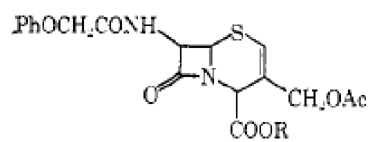
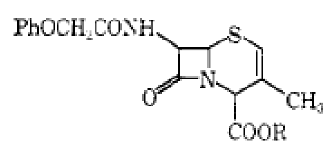
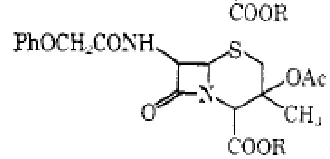
Calculating the theoretical wavenumber for a range of β-lactam structures can be useful in identifying which ones are likely to have useful activity before synthesizing them.

β-lactam antibiotics

The infrared frequency of the **β-** lactam can be used as an indicator of acylating power (the higher the frequency the better the acylating agent). The data in Table II suggest a rough but positive correlation between acylation ability and biological activity.

However, a strained **β**-lactam, as indicated by high IR frequencies, need not be reactive…

Table II

Compound	β-Lactam frequency,[a] cm^{-1}	Bioassay[b]
	1790	1800
	1795	High
	1792	300
	1785	25
	1776	4
	1784	6
	1780	15
	1780	Low

JACS 91 1401 (1969).

[a] Determined in $CHCl_3$ solution on the methyl esters ($R = CH_3$).
[b] Assay on the salts in Oxford units against a penicillin G sensitive *Staphylococcus aureus* strain.

S. Sun, Advanced Materials 18, 393 (2006).

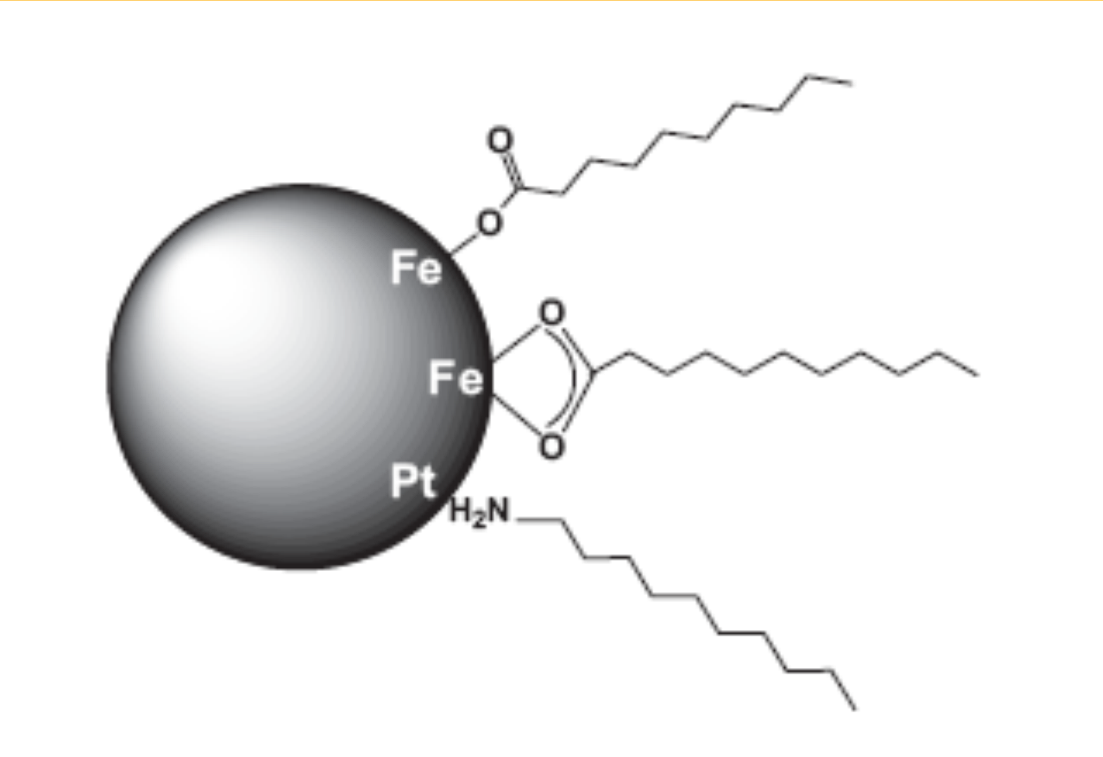


Figure 7. Schematic of binding of alkyl carboxylate and alkylamine molecules to a FePt nanoparticle.

FePt nanoparticles are generally stabilized with alkyl carboxylic acid (RCOOH) and alkylamine (RNH2). –COOH can covalently link to Fe, forming iron carboxylate (-COO–Fe). On the other hand, –NH2, as an electron donor, prefers to bind to Pt via a coordination bond. Detailed IR spectroscopy studies on FePt nanoparticles coated with oleic acid and oleylamine indicate the presence of both –NH2 and –COO– on the nanoparticle surfaces, as shown in Figure 7. The –COO– acts either as a chelate ligand, binding to Fe via two O atoms, or as a monodentate molecule, linking to Fe via only one O atom.

Low frequency normal modes in proteins

Current Opinion in Structural Biology *2005*, **15**:586–592

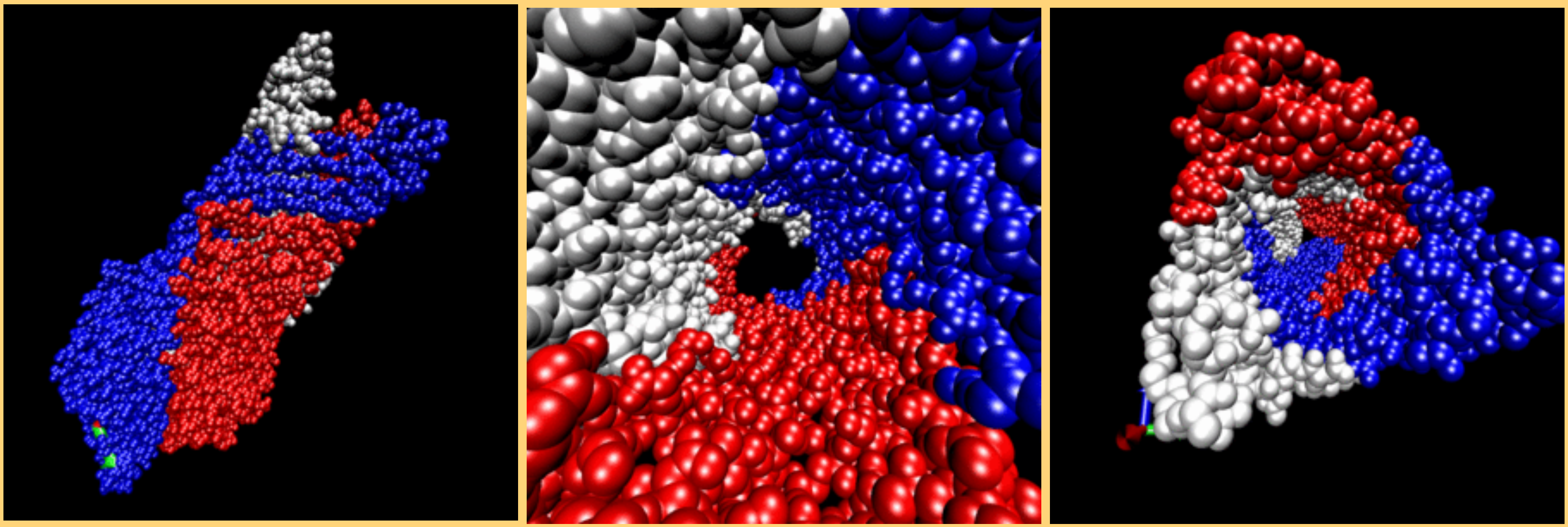
Recent advances in sequencing and structural genomics indicate that the canonical sequence-to-structure-to-function paradigm is insufficient for understanding and controlling the mechanisms of biomolecular interactions and functions.

Because molecular structures are dynamic rather than static, information regarding their dynamics is required to establish the link between structure and function. Normal mode analysis (NMA) has re-emerged in recent years as a powerful method for elucidating the structure-encoded dynamics of biomolecules.

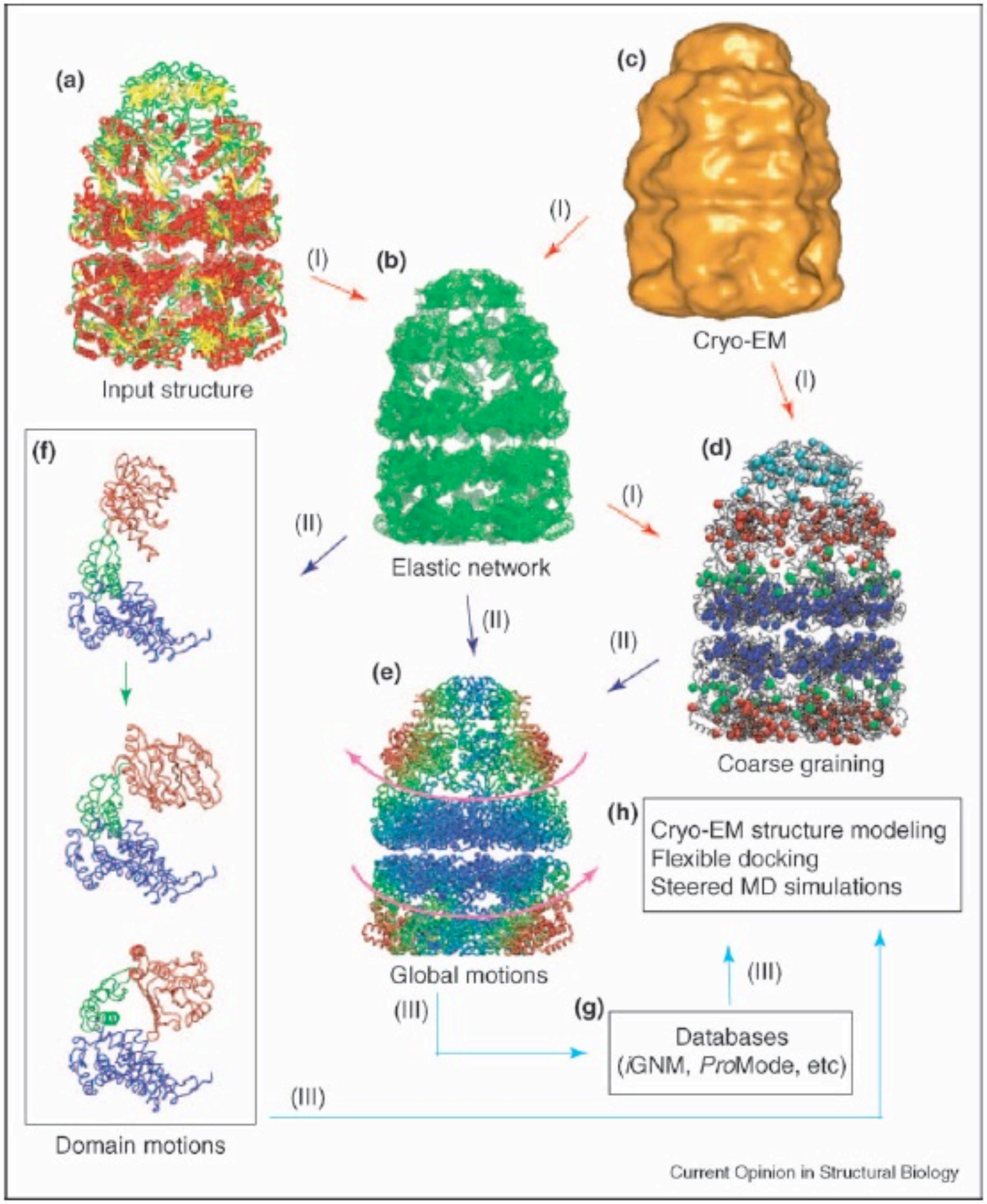
It is plausible that the motions NMA predicts are functional if one considers that each protein functions only if it is folded into its equilibrium/native structure and that each equilibrium structure encodes a unique equilibrium dynamics. Furthermore, NMA yields a unique analytical solution of the modes of motion accessible at equilibrium (near a global energy minimum). Thus, the equilibrium dynamics predicted by NMA, and the structure-encoded collective motions in general, ought to be functional, based on the premise that each protein has evolved to optimally achieve its biological function.

E. coli membrane channel protein TolC

Putative TolC opening/closing. TolC is a homo-trimer. Each monomer is indicated by a separate color.



Low frequency normal modes in proteins often have biological significance


(a)
Input structure
(I)
(b)
Elastic network
(I)
(c)
Cryo-EM
(I)
(d)
Coarse graining
(I)
(f)
Domain motions
(II)
(II)
(II)
(e)
Global motions
(h)
Cryo-EM structure modeling
Flexible docking
Steered MD simulations
(III)
(III)
(g)
Databases
(iGNM, ProMode, etc)
(III)
Current Opinion in Structural Biology