UV- Absorption Spectrophotometry.

The UV light is absorbed by various compound, namely by those having conjugated double bonds.

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Both proteins and nucleic acids absorb strongly UV-light, which can be used for their investigation.

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The a.a. tryptophan and tyrosine have absorption maxm at about 280nm. Phenyl-alanine at 255nm.

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Nucleotides (nitrogen bases) have absorption maxm in the range of 260-270nm.

Chromophores (are the moiety) – their absorption properties vary according to chemical composition of the medium.

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Molecules containing functional groups, capable of absorbing UV/VIS radiation are called chromophores.

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Electron jumps possible π to π^*, n to π^*.

IR. / Vibrational spectroscopy bcz it helps to detect vibration in a molecule.

IR interact with rotational and vibrational states of molecules.

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Complex molecules can vibrate or rotate in many different ways (modes).

↓

Various chemical groups ($-CH_3$, $-OH$, $-COOH$, $-NH_2$) etc have specific vibration and rotation frequencies and thus

IR spectrum is unique for every molecule.

NIB:- Exact structure of carbohydrate can be given be IR, NMR or GCMS.

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absorb IR light of specific λ.

↓

For Nucleic acid, UV-absorption and X-ray.

Therefore, infrared absorption spectra have many maxima. A change in chemical structure is manifested as changes ~~chan~~ of the position of ~~the~~ maxima.

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For Protn NMR, IR, X-ray Crystallography Can be used

IR Spectroscopy is also known as vibrational spectroscopy since it causes vibrational transitions.

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The vibrations in the IR spectroscopy is known as fundamental vibrations.

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IR Spectrum is mainly used in structural elucidation to determine the functional groups.

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The IR region of the spectrum encompasses radiation with wavenumbers ranging from about 12,800 - 10 cm^{-1}.

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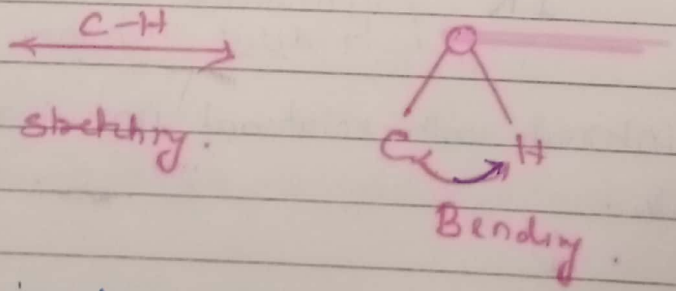
There are 2 kinds of vibration:-

(1) Stretching (2) Bending.



Stretching involves changes in length of inter-atomic bond.

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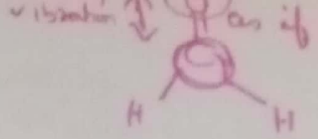
Bending involve change in angle between 2 bonds.



Carbonyl functional group

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A more modified form of IR is FTIR.

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In IR, grating is used whereas in FTIR we use a moving mirror.

Dispersion Spectrometry / IR

In order to measure an IR spectrum the dispersion spectrometer takes several minutes

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Also the detector recieves only a few % of the energy of original light source.

FTIR

In order to measure an IR spectrum, FTIR takes only a few seconds

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Moreover the detector recieves upto 50% of the energy of original light source (much larger than the dispersion spectrometer).

↓

In Infrared spectra -

(1) Position of band depends on -

Mass of atoms

Light atoms give high frequency.

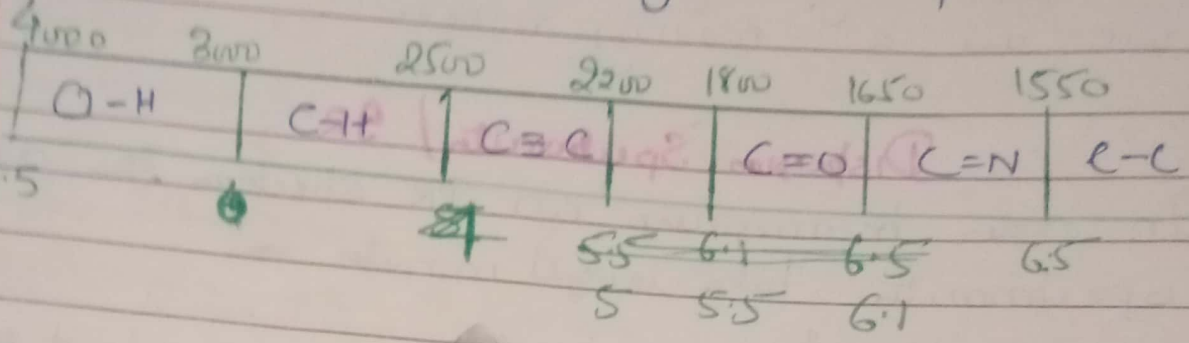
Bond strength

Strong bonds give high frequency.

(2) Strength of band depends on -

Change in dipole moment i.e. A large change in dipole moment gives strong absorption.

Width of band depends on -
- Hydrogen bonding.
Strong H-bond gives wide peak.



Questions.

To check if a protein is phosphorylated, you would perform

1) Southern blot.
2) Chip assay
3) Immuno
4) Western blot.

Fusion Partner with ligand:-
Partner — Ligand
Streptavidin — Biotin
6-Histidine tag — Nickel
Maltose binding protein — Amylose
Glutathione-S-transferase — Glutathione
Flag tag — Specific Antibody