X-ray Crystallography, IR Spectroscopy, Atomic Absorption Spectroscopy, UV-Vis Spectroscopy, and Fluorescence


**SPECTROSCOPY**

- **Definition:** Interaction of energy with matter in order to deduce structural information about a compound
  - Gamma rays > x-rays > UV light > visible light > IR light > microwaves > radio waves
  - X-ray measures inner electrons
  - UV/Vis region measures outer electrons
  - IR measures molecular vibrations (the way bonds bend and stretch)
  - Microwave region measures molecular rotations
  - Radio region measures nuclear spin
- **Absorption:** Shine light on a sample and measure the intensity of the light after it interacts with that sample
  - System absorbs the energy, emits it, and you get a spectrum
- Given a sample, can use chromatography to isolate it and then spectroscopy to identify it
  - IR, UV-Vis, and MS can help identify class of the compound and molecular weight (MS)
  - NMR needs to be done after IR, UV-Vis or MS in order to fully elucidate the structure
  - X-ray can directly identify the isolated compound (without need for NMR)

**XRAY CRYSTALLOGRAPHY**

- Uses **diffraction** (bending) of light around **crystal** of a pure compound
- These crystals have a repeating structure which can be visualized with x-ray diffraction and used to identify the compound
- Can be used for small (100-2,000 Da) or large (>2,000 Da) molecules
  - **Strength:** Able to identify entire structure (and stereochemistry) for a molecule
  - **Weakness:** Is extremely laborious and difficult to get a pure crystal
**IR SPECTROSCOPY**

- Bonds have specific **vibrations** associated with them, and IR spec can identify these vibrations.
- Molecule will absorb IR radiation that matches its molecular vibrations.
- IR spectrum readout: X-axis is wavenumber and y-axis is % transmittance.
- **Strength** – reveals functional groups present in a compound.
- **Weakness** – tells you nothing about how these functional groups are connected.

**ATOMIC ABSORPTION SPECTROSCOPY**

- Flame is used to convert metal ions to atoms.
- Atom absorbs a specific wavelength of light and will emit this light when the excited electron relaxes.
- Each element has a unique absorption and emission spectrum.
- **Weakness** – you destroy the sample when you do this, there is a low throughput.
- Not used too often anymore.

**UV-VIS SPECTROSCOPY**

- Molecules absorb electromagnetic radiation
  - Electrons go from low-energy state to high-energy state.
- Some molecules can pass through the detector unnoticed → organic molecules (glucose, certain fats, etc.)
  - In the 190-800 nm region.
- **Chromophore** = functional group that absorbs UV radiation
  - Extended conjugation is KEY for a molecule to be a good chromophore.
  - Conjugation = aromatics or double bonds adjacent to each other.
- UV detectors can only look at one wavelength.
- Photodiode array (PDA) detectors can scan all wavelengths → still need extensive UV fingerprint to get any useful information.
- The electromagnetic absorption spectrum can serve as a molecule fingerprint when compared to databases of other molecules.
- **Dereplication** = can rapidly identify a known chemical scaffold
  - Chemical scaffold – has a core structural unit that is unique to a certain class of molecules.
- **Strength** – easy to perform, can reveal the class of the molecule.
- **Weakness** – need an extensive spectrum (a good chromophore). Can’t identify certain organic molecules. Can’t distinguish between isomers of same molecular class.
Can derivatize a molecule to make it UV active but have to be careful because then you’re changing the structure of the molecule.

- **Evaporative Light Scattering Device (ELSD)** = can be used instead of or in addition to UV/PDA detector
  - Uses a nebulizer to evaporate solvent from an analyte
  - All that matters is if the compound is present or not → did something cross the laser or not
    - Really sensitive and requires little material but does destroy the sample
  - **DOES NOT require a chromophore to be present**

**FLUORESCENCE**

- Electron is excited by absorption and then emits fluorescence upon relaxation
- Stokes shift = difference between excited and emitted wavelengths
- **Fluorophore = molecules or functional groups that have the capacity to exhibit fluorescence**
  - Require extended conjugation of pi bonds
  - More conjugated → less energy required for excitation → longer wavelength can be used for excitation
- Fluorescent probes used to identify biological processes
  - **Green fluorescent protein (GFP)** – fluoresces green light when exposed to light in the blue to UV range
    - Can make its own color using oxygen only
    - Slight modifications can allow for different colors to be emitted
Hiyoungh performed IR spectroscopy on the molecule below. She will be informed of all of the following except:

A. Indicate a C-S bond
B. The placement of NH/NH₂ groups
C. The presence of amide functionality
D. Indicate C-H, C=H bonds

Ampicillin

Antonio would like to open up his own biotech startup company that analyzes the presence of common “labs” compounds in liquid (serum, blood, etc). He obtained pure standards of these molecules. Upon HPLC analysis (assuming each is run under appropriate conditions), which molecule(s) would require ELSD detection?
Farah can use UV fingerprints to distinguish between the following two analogs of the antibiotic ampicillin:

A. True
B. False

Farah can use UV fingerprints to distinguish between the following two analogs of the antibiotic resistomycin:

A. True
B. False
Farah can use UV fingerprints to distinguish between the following two azaphilone antibiotics:

A. True

B. False