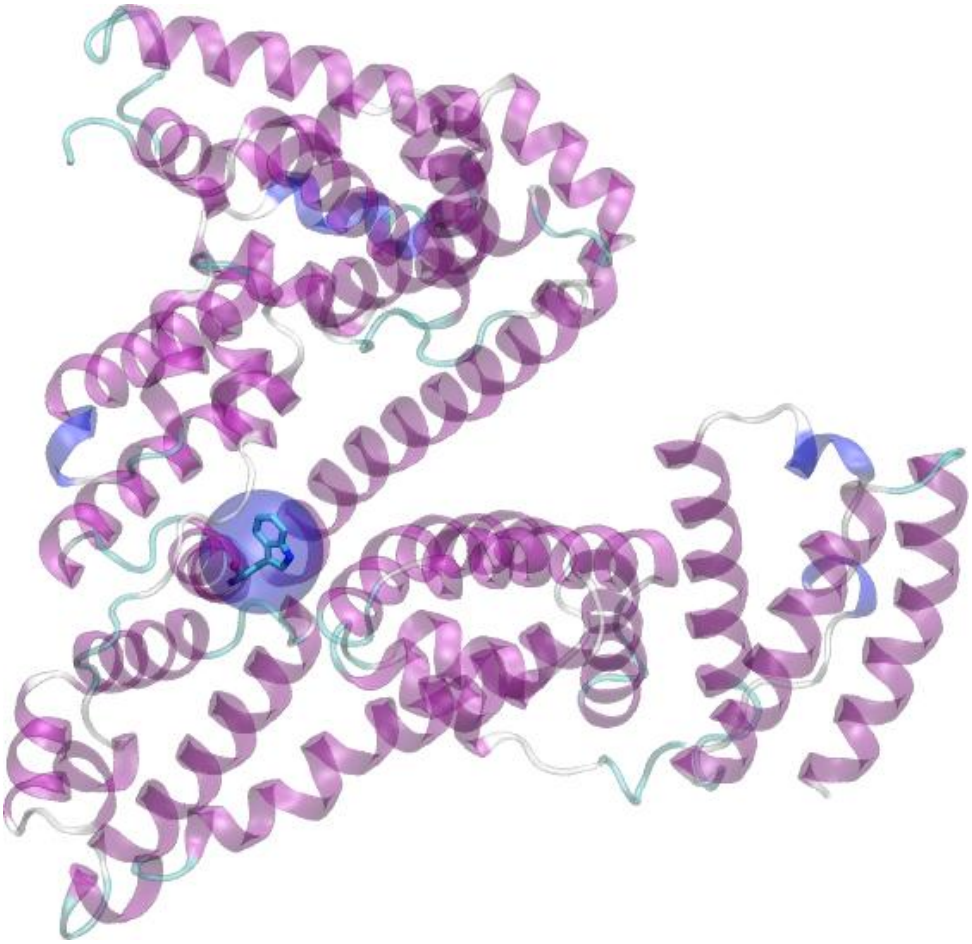
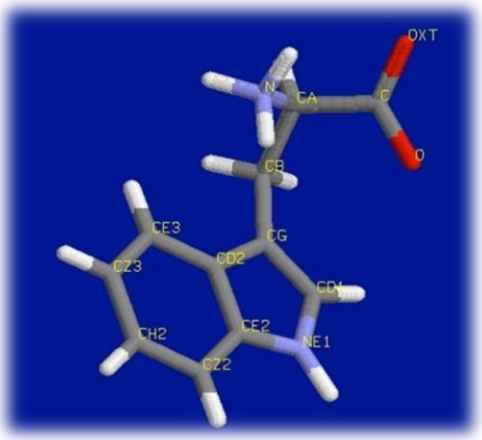
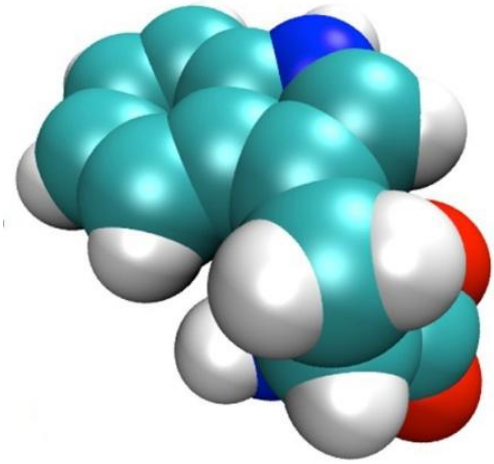
Molecular Environment Sensitivity of Tryptophan





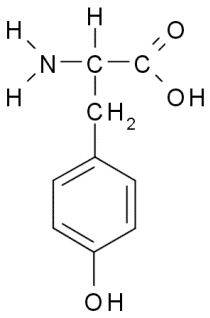


The Hand-in Assignment

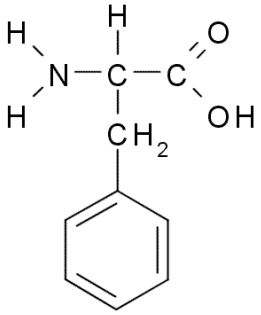
Mengshu Hao

1. Which other amino acids than tryptohan display fluorescence emission?

Tyrosine & Phenyl Alanine



Try



Phe

2. Why does an absorption spectrum of a molecule very often display several peaks whereas the fluorescence emission spectrum only contains one single peak?

Because there are always only emission of excited electron from S_1- S_0 in fluorescence emission spectrum. Whereas absorption spectrum has several transitions, e.g. S_0- S_1, S_0- S_2.

3. Why was 295 nm chosen as excitation wavelength during the recording of tryptophan fluorescence emission spectra?

Different samples will have different fluorescence emission spectra. The fluorescence of a folded protein is a mixture of the fluorescence from individual aromatic residues. Most of the intrinsic fluorescence emissions of a folded protein are due to excitation of tryptophan residues, with some emissions due to tyrosine and phenylalanine; but disulfide bonds also have appreciable absorption in this wavelength range. Typically, tryptophan has a wavelength of maximum absorption of 280 nm and an emission peak that is solvatochromic, ranging from ca. 300 to 350 nm depending in the polarity of the local environment. Hence, protein fluorescence may be used as a diagnostic of the conformational state of a protein.

At 295 nm, the tryptophan emission spectrum is dominant over the weaker tyrosine and phenylalanine fluorescence. So we chose 295nm as excitation wavelength during the recording of tryptophan fluorescence emission spectra.

4. Fluorescence spectroscopy is a much more sensitive technique when investigating local molecular environments compared to UV spectroscopy. Why is that?

When performing experiments with denaturants, surfactants or other amphiphilic molecules, the microenvironment of the tryptophan might change. For example, if a protein containing a single tryptophan in its 'hydrophobic' core is denatured with increasing temperature, a red-shift emission spectrum will appear. This is due to the exposure of the tryptophan to an aqueous environment as opposed to a hydrophobic protein interior. In contrast, the addition of a surfactant to a protein which contains a tryptophan which is exposed to the aqueous solvent will cause a blue shifted emission spectrum if the tryptophan is embedded in the surfactant vesicle or micelle. Emission is susceptible about the solvent, but not for the absorption. That's why fluorescence spectroscopy is more sensitive then UV spectroscopy.

5. What is the definition of the quantum yield and what are typical fluorescence lifetimes of biological fluorophores?

Quantum yield

The fluorescence quantum yield gives the efficiency of the fluorescence process. It is defined as the ratio of the number of photons emitted to the number of photons absorbed.

$$\text{Quantum Yield} \quad \Phi = \frac{photons\ emitted}{photons\ absorbed}$$

The maximum fluorescence quantum yield is 1.0 (100%); every photon absorbed results in a photon emitted. Compounds with quantum yields of 0.10 are still considered quite fluorescent.

Fluorescent lifetime

Conjugated dyes generally have a lifetime between 1-10 ns, a small amount of longer lived exceptions exist, notably pyrene with a lifetime of 400ns in degassed solvents or 100ns in lipids and coronene with 200ns. The fluorescent lifetime for human serum albumin (HSA) is 50ns, Coumarin 6 is 2.5ns, DAPI + ssDNA is 1.88ns, DAPI + dsDNA is 2.20ns, Ethidium Bromide + ssDNA is 25.1, Ethidium Bromide + dsDNA is 28.3, Pyrene is more than 100ns, and so on.

6. What is fluorescence quenching?

Quenching refers to any process which decreases the fluorescence intensity of a given substance. A variety of processes can result in quenching, such as excited state reactions, energy transfer, complex-formation and collisional quenching. As a consequence,

quenching is often heavily dependent on pressure and temperature. Molecular oxygen and the iodide ion are common chemical quenchers.

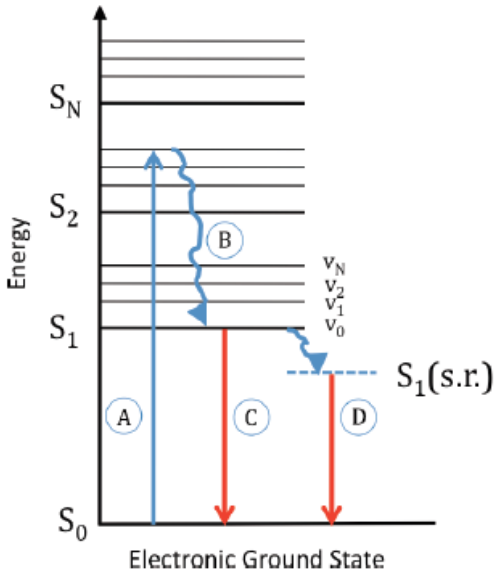
7. How can one use fluorescence quenching as a tool in the investigation on molecular interactions in biochemical and biophysical related problems?

Molecular interaction can be reflected from the shift in the fluorescence emission. Fluorescence quenching titration is a sensitive and rapid method for the study of steroid-protein molecular interactions. The procedure has been applied to systems containing androstane derivatives and proteins or aromatic amino acids.

For example, testosterone binding may alter bovine serum albumin (BSA) conformation and that the measured binding capacity of this protein for testosterone is dependent on the protein concentration. High molar ratios of bound steroid to protein are obtained with dilute albumin solutions and may reflect the N-F transformations observed by others.

8. Why is typically fluorescence spectra recorded in polar environments more red-shifted compared to non-polar environments?

Increasing the polarity of the solvent can make more red-shift. Because the polarization of the solvent can decrease the energy of the excited state (e.g., S_1 have different level, v0, v_1, v_2, v_N…), then make the gap between the base state and excited state smaller. Then the red-shift enhance.

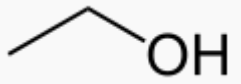
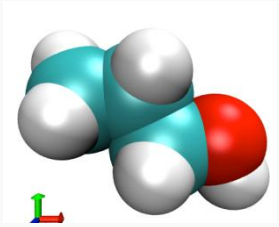
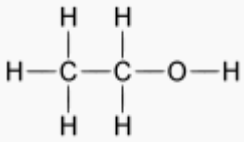
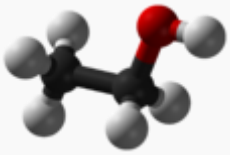


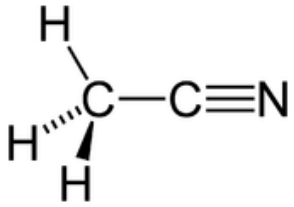
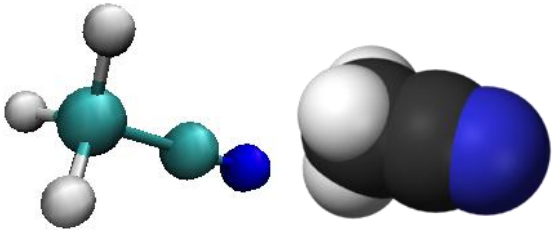
9. What are the underlying principles for using reporter molecules such as tryptophan as a molecular reporter on conformational changes in proteins?

The fluorescence of a folded protein is a mixture of the fluorescence from individual aromatic residues. Most of the intrinsic fluorescence emissions of a folded protein are due to excitation of tryptophan residues, with some emissions due to tyrosine and phenylalanine; but disulfide bonds also have appreciable absorption in this wavelength

range. Tryptophan fluorescence is strongly influenced by the proximity of other residues (i.e., nearby protonated groups such as Asp or Glu can cause quenching of Trp fluorescence). Also, energy transfer between tryptophan and the other fluorescent amino acids is possible, which would affect the analysis, especially in cases where the Förster acidic approach is taken. In addition, tryptophan is a relatively rare amino acid; many proteins contain only one or a few tryptophan residues. Therefore, tryptophan fluorescence can be a very sensitive measurement of the conformational state of individual tryptophan residues. The advantage compared to extrinsic probes is that the protein itself is not changed. The use of intrinsic fluorescence for the study of protein conformation is in practice limited to cases with few (or perhaps only one) tryptophan residues, since each experiences a different local environment, which gives rise to different emission spectra.

10. Please present models and physical properties for the solvents studied:
Create the molecules (exchange buffer for water) and present them as 3D models (e.g. through PRODRG – server + VMD). Report also solvent polarities in terms of dielectric constants at 20 degrees Celsius.

Ethanol	
	
	
Properties	
Molecular formula	C_2H_6O
Molar mass	46.07 g mol^{-1}
Exact mass	46.041864814 g mol^{-1}
Appearance	colourless liquid
Density	0.789 g cm^{-3} (at 20 °C)
Melting point	−114 °C, 159 K, -173 °F
Boiling point	78 °C, 351 K, 172 °F
Solubility in water	miscible
Acidity (pK_a)	15.9[1]
Refractive index (n_D)	1.36
Viscosity	1.200 cP (1.200 mPa·s) (at 20 °C)
Dipole moment	1.69 D (gas)

Acetonitrile	
	
	
Properties	
Molecular formula	C_2H_3N
Molar mass	41.05 g mol^{-1}
Appearance	colorless liquid
Density	0.786 g/mL liquid
Melting point	–45 °C
Boiling point	82 °C
Solubility inwater	miscible
Solubility	organic solvents
Acidity (pK_a)	25

Polarities:

Ethanol is much less polar than water; its dielectric constant is 24.3 (at 25 °C).

Acetonitrile's dielectric constant is 37.5 (at 20°C).

Water's dielectric constant is 80.1(at 20°C).

Polarity: Water > Acetonitrile > Ethanol

Results from experiments:

These showed that the different solution has different stokes shift. The more polarity the solution is, stokes shift will be more obviously.

So,

Polarity: Water > Acetonitrile > Ethanol

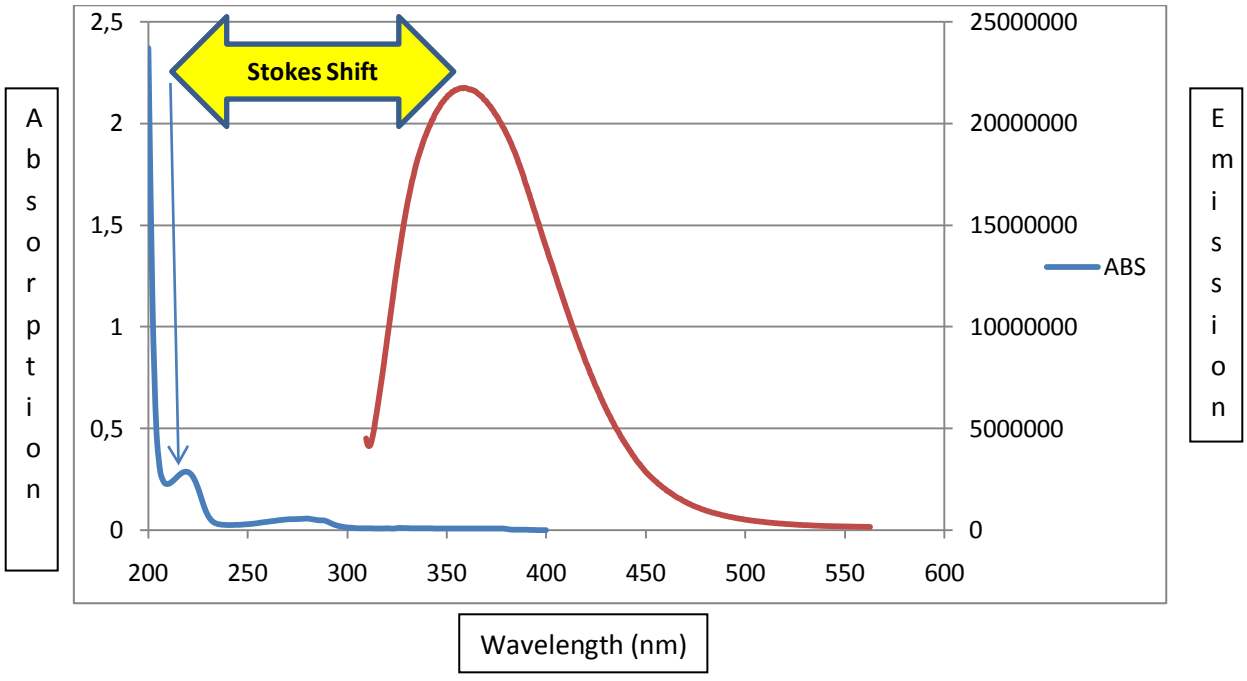
Stokes shift: Water > Acetonitrile > Ethanol

And,

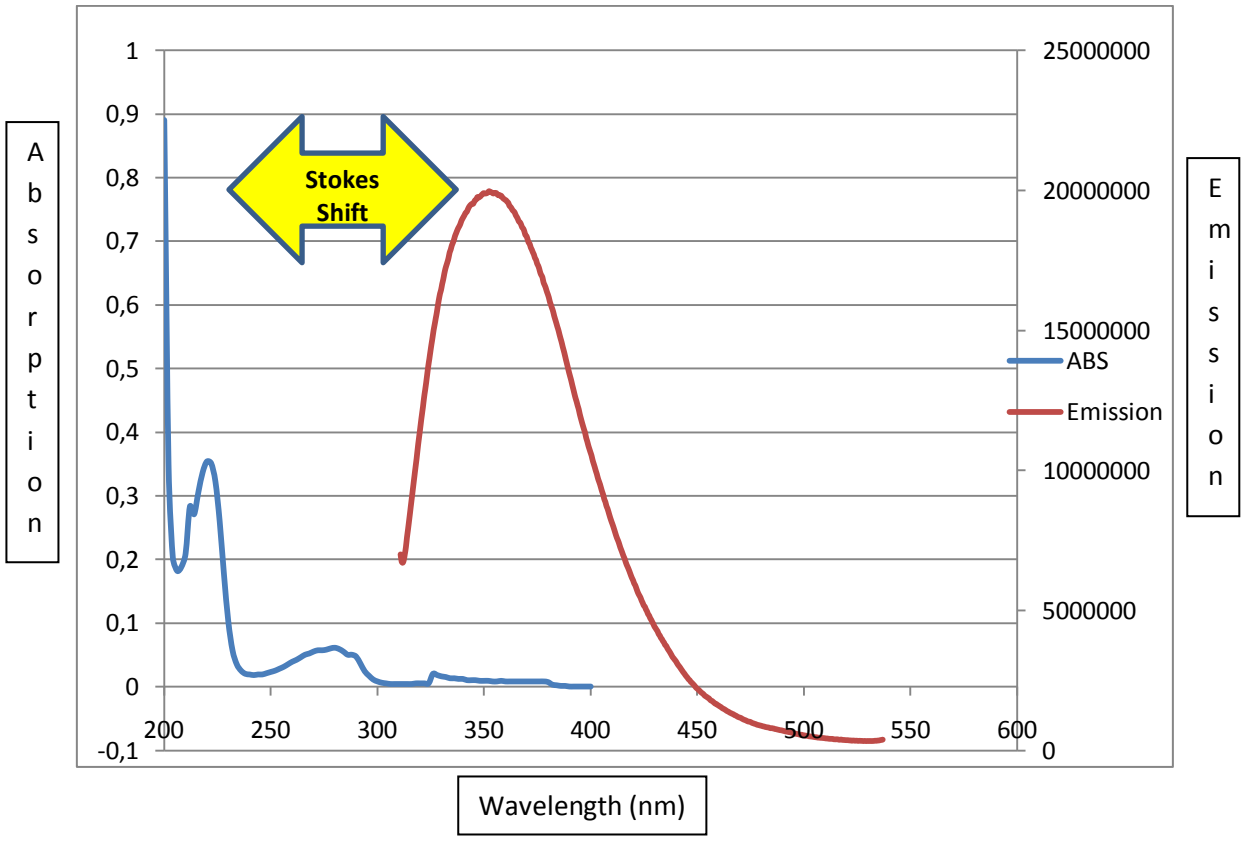
we know that fluorescence emission spectroscopy is steady state.

Co-worked Graphs

Stokes shift (Tryptophan in Pure Buffer)



Stokes shift (Tryptophan in Acetonitrile)



Stokes shift (Tryptophan in Ethanol)