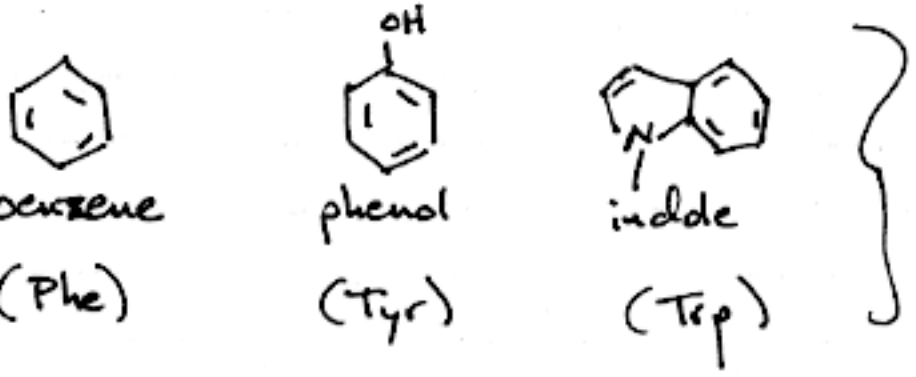
12/3/01

Protein and Nucleic Acid Chromophores

Particle in a box tells us to get lower E (longer wavelength) transitions, we need a "bigger box."

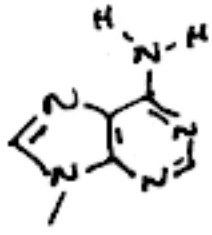
For carbon-based systems, the way to get a big box is conjugated π-systems.

e.g.



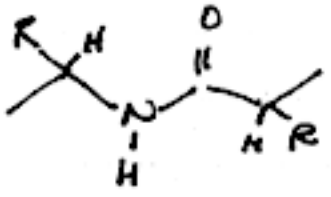
benzene (Phe) — phenol (Tyr) — indole (Trp) } All around 250-280 nm

$\pi \rightarrow \pi^*$

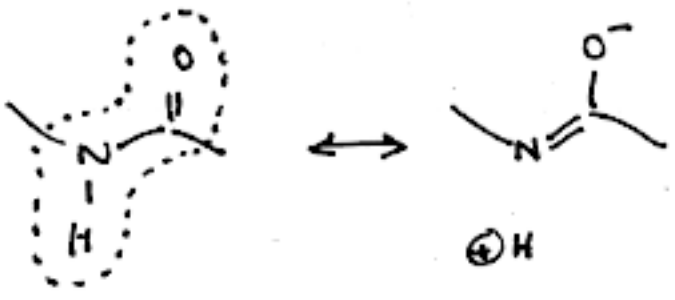


Adenine and G, C, T $\lambda_{MAX} \approx 250\text{-}260$

Smaller "box" peptide bond



190 ~~200~~-230 nm



sp^2 hybrid carbons, nitrogens, oxygens

See real protein spectra on 548-551

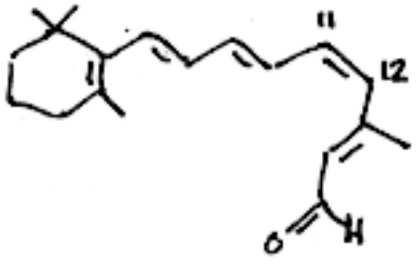
Complex combinations of Trp, Phe, Tyr

Note that even pure Trp has complex spectra.
⇒ multiple transitions $\pi_1 \rightarrow \pi_1^*$ $\pi_2 \rightarrow \pi_2^*$ etc.

And in a protein, each Trp is in a slightly different environment. This effects energy of $e^{\ominus}$'s in each orbital (alters ψ_i), leading to various ΔE's.

→ Skip Hyper/Hypochromicity.

Rhodopsin (p. 553-554)

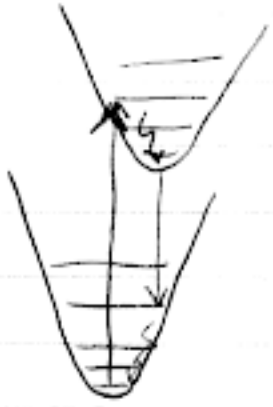


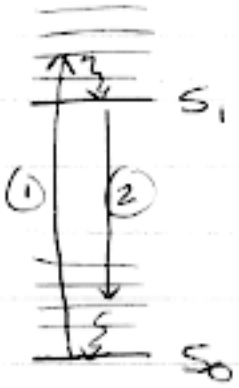
Absorption of light induces cis→trans isomerization. Then signal gets sent to our brain

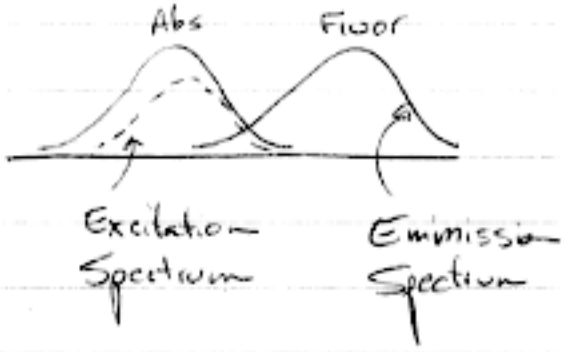
λ_{max} = 440-565nm

depending on protein environment (charged amino acids here or there) → visual cones, RGB, all use same chromophore

Fluorescence & Phosphorescence pp. 554-565





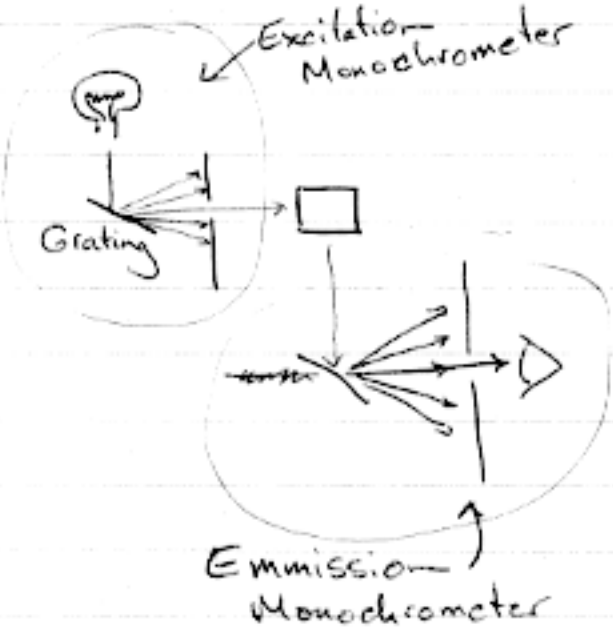


(1) **Excitation Spectrum** –

Sit at one emission wavelength and scan excitation.

→ often same as the absorption spectrum, but not necessarily.

Q: Why?

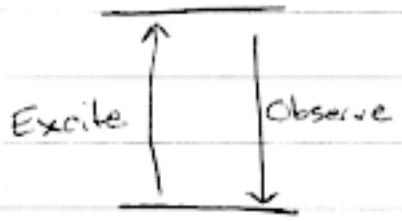


(2) **Emission Spectrum** –

Sit at one excitation wavelength and scan emission

These tell us about local environment.

Lifetime measurements



If we excite the transition and then turn off the excitation light, the emission light will decay, as the excited state depopulates.

Usually 1ST order

$$-\frac{d[S_1]}{dt} = k_d[S_1]$$

S_1 = excited state. Called M* in the book.

$$F \propto [S_1]$$

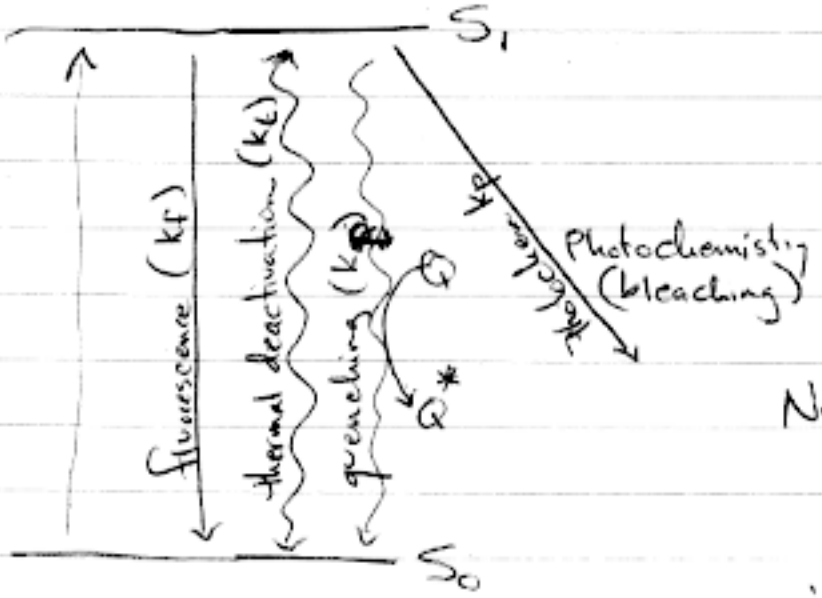
$$[S_1] = [S_1]_0 e^{-t/\tau_0}$$

AS FOR KINETICS

$$= [S_1] e^{-k_d t}$$

$$\tau = \frac{1}{k_d}$$

$\tau_0 \equiv$ fluorescence lifetime



$k_d \equiv$ all decay paths

$$k_d = k_f + k_t + k_Q + k_p$$

Note: $k_d = 1/\tau_0$ $k_f = 1/\tau_0$

Note k_Q may depend to 1ST order on a quenching species.

ie. really $k_Q[Q]$