Circular Dichroism
Fluorescence
Molecular Biology

How they work.
What they can do.
Where are they useful.
What are requirements.

Circular dichroism
Circular dichroism means that RCP and LCP light have different extinction coefficients. One is absorbed more than the other. Di Chroism: two absorbances.
Here the green vector has been absorbed upon passage through the material. The resulting light is elliptically polarized, and its major axis remains parallel to the plane of polarization that entered the medium.

http://www.enzim.hu/~szia/cddemo/edemo14.htm
Circular birefringence (= optical rotation)

If RCP and LCP have different refractive indices, so that one is delayed more than the other, the axis that bisects them is rotated.

bi refringence = two refractive indices.

http://www.enzim.hu/~szia/cddemo/edemo15.htm

Circular dichroism is also related to ellipticity via the difference between the major and minor axes of the ellipse that results.

Previously plane-polarized light emerges from the sample elliptically polarized.

The amount of ellipticity can be described by the angle $\Theta$

$\Theta = \Delta \varepsilon \times 3298.2$

$\Theta$ is the molar ellipticity.

Circular dichroism

$$\Delta \varepsilon = \varepsilon_L - \varepsilon_R$$

$\varepsilon$ is a function of $\lambda$ and so is $\Delta \varepsilon$.

$\Delta \varepsilon$ can be either negative or positive (vs. $\varepsilon$ which is always positive).

CD only occurs near an absorption band (i.e. $\varepsilon \neq 0$)

It also only occurs for molecules that are ‘optically active’.

Optical activity: a molecule cannot be superimposed on its mirror image.

Opposing enantiomers have oppositely signed CD.
Anticipated results: the CD spectrum of ovalbumin

After cleavage of the backbone, here is the other structure this mutant ovalbumin can adopt. Find the difference!

Advantages: more selective, combines and emission spectrum and an absorption spectrum, observed on a zero background.

Emission is observed at a 90° angle from the incident beam to separate it from transmitted light.

Fluorescence (red-shifted).
We minimize background by centrifuging or filtering out anything that could scatter.

The change in intensity may be slight.
Anthracene’s vibrationally-resolved electronic spectra.

**Figure 1.8.** Mirror-image rule and Franck-Condon factors. The absorption and emission spectra are for anthracene. The numbers 0, 1, and 2 refer to vibrational energy levels. From [11].

From Lakowicz’ Book “Principles of Fluorescence Spectroscopy”


Fluorescence takes place on a much longer time scale than absorption, so it is sensitive to a much wider range of interactions and perturbations.
Fluorescence Quantum Yield

\[ \varphi_F = \frac{k_F}{k_{IC} + k_{IS} + k_{Q} Q + k_F} \]

Fraction of excited state that returns to the ground state via fluorescence. This is the same as the fraction of photons absorbed that lead to fluorescence.

\[ k_F = \frac{\lambda_{10}}{} \]

\( k_F \) is the intrinsic fluorescence rate constant. Radiative lifetime \( \tau_F = 1/k_F \)

Internal conversion: \( k_{IC} \) non-radiative decay, mediated via collisions with solvent, internal motions (vibrations). Increases with temperature.

Intersystem crossing: \( k_{IS} \) electron spin flip to a triplet state (different manifold of states). Inefficient (forbidden) slow and usually masked by other processes. Usually need low T, rigid glass and low \([O_2]\).

Quenching: \( k_{Q} Q \) dissipation of energy via interactions with other molecules, or functionalities. Since the natural lifetime of most organic fluorophores is only 1 - 100 ns, only efficient quenchers are significant. I, O_2, acrylamide

Because fluorescence emission is a slower process, it is more sensitive to the environment and interactions of the chromophore. This makes it more useful.

1-Anilinonaphthalene-8-Sulfonic Acid (ANS)

The lower polarity environment in the core of a protein is less quenching of fluorescence and less supportive of internal conversion.
Tryptophan fluorescence differs depending on exposure to solvent. It therefore reports on protein unfolding.

The Stokes shift is larger in a more polar solvent because the energies associated with interactions with polar solvent molecules are larger.

Figure 16.11. Effect of tryptophan environment on the emission spectra. The emission spectra are those of apoazurin Pfl, ribonuclease T1, staphylococcal nuclease, and glucagon, for 1 to 4, respectively. Revised from [59] and [60].

Figure 16.11 from Lakowicz

Quenching of tryptophan in proteins

Buried (blue-shifted) tryptophan is less accessible to polar quenching agents, so the quenched spectrum is blue-shifted.

Figure 16.30. Collisional quenching of buried (W1) and surface accessible (W2) tryptophan residues in protein.

Figure 16.30 from Lakowicz
The Stern-Volmer Equation for Quenching

In the presence of quencher
\[ \varphi_F = \frac{k_F}{k_{IC} + k_{IS} + k_F + k_Q[Q]} \]

The ratio of fluorescence in the absence of Q vs. in the presence of Q is
\[ \frac{F_0}{F} = 1 + k_Q[Q] \tau \]

The Modified Stern-Volmer Equation

Observed fluorescence is the sum of fluorescence from exposed sides and buried sites.
\[ F = F_X + F_B \]

For each type of site: \[ F = \frac{F_0}{1 + k_Q[Q] \tau} \]

Slope
\[ \frac{F_0}{\Delta F} = \frac{1}{f_X k_Q \tau} + \frac{1}{f_X} \]

Intercept yields \( f_e \), the fraction of fluorescence due to exposed sites.

\( F_0 \) is the total fluorescence when there is no quencher.

Dependance on quencher concentration: Stern-Volmer plots

Figure 16.36 from Lakowicz

Figure 16.36: Stern-Volmer and modified Stern-Volmer plots for apomyoglobin quenching by iodide or trichloroethanol (TCE). The data in the upper panel was reconstructed from the data in the lower panel. Data from [113].