

An Introduction to Fluorescence Polarization

The theory of **Fluorescence Polarization**, first described in **1926 by Perrin**, is based on the observation that fluorescent molecules in solution, excited with plane-polarized light, will emit light back into a fixed plane (i.e. the light remains polarized) if the molecules remain stationary during the excitation of the fluorophore. Molecules, however, rotate and tumble, and the planes into which light is emitted can be very different from the plane used for initial excitation.

The polarization of a molecule is proportional to the molecule's rotational relaxation time, or the time it takes to rotate through an angle of 68.5° . Rotational relaxation time is related to viscosity, absolute temperature, molecular volume and the gas constant.

Principle: When a fluorescent molecule is excited with plane polarized light, light is emitted in the same polarized plane, provided that the molecule remains stationary throughout the excited state (which has a duration of 4 nanoseconds for fluorescein). If the molecule rotates and tumbles out of this plane during the excited state, light is emitted in a different plane from the excitation light. If vertically polarized light is exciting the fluorophore, the intensity of the emitted light can be monitored in vertical and horizontal planes (degree of movement of emission intensity from vertical to horizontal plane is related to the mobility of the fluorescently labeled molecule). If a molecule is very large, little movement occurs during excitation and the emitted light remains highly polarized. If a molecule is small, rotation and tumbling is faster and the emitted light is depolarized relative to the excitation plane.

Definition of Fluorescence Polarization:

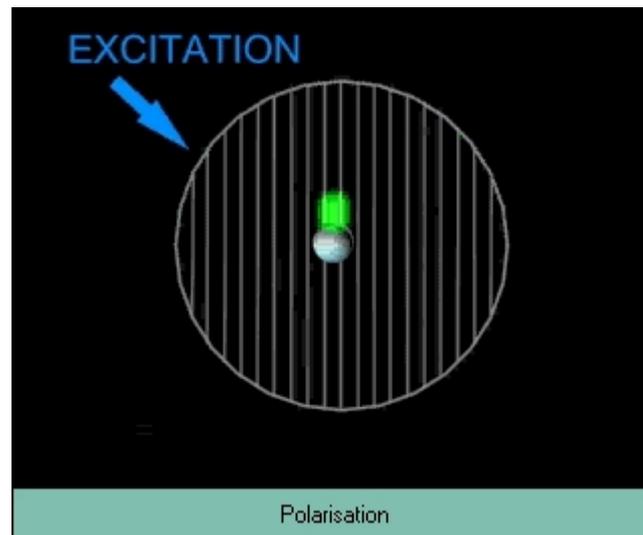
$$P = \frac{I - I_{\perp}}{I + I_{\perp}}$$

I ... Intensity with polarizers parallel

I_⊥ ... Intensity with polarizers perpendicular



See below a video clip describing the principle of FP:



Explanation: Small molecules rotate quickly during the excited state, and upon emission, have low polarization values. Large molecules, caused by binding of a second molecule, rotate little during the excited state, and therefore have high polarization values.

Applications for Fluorescence Polarization

Fluorescence Polarization is a technique specially applied to study molecular interactions. It gives a direct, nearly instantaneous measure of a tracer's bound/free ratio.

FP experiments are done in solution without solid supports, allowing true equilibrium analysis down to the low picomolar range. FP measurements do not adulterate samples, so they can be treated and reanalyzed in order to ascertain the effect on binding by changes such as pH, temperature, and salt concentration. Additionally, FP experiments are taken in "real-time" and experiments are not limited to equilibrium binding studies.

Overview of application areas

- Receptor/ligand studies (e.g. hormone/receptor assays)
- Protein/peptide interactions
- DNA/protein interactions
- Tyrosine Kinase Assays
- Competitive Immunoassays

Advantage of FP

Fluorescence Polarization offers numerous advantages over more conventional methods to study the binding of proteins to nucleic acids (particularly in that no hazardous radioactive waste is generated) and has a lower limit of detection in the sub-nanomolar range. FP is furthermore truly homogeneous, allows real-time measurements (kinetic assays), is insensitive to variations in concentrations and is an optimal solution for homogeneous assay formats (no separation by washing).