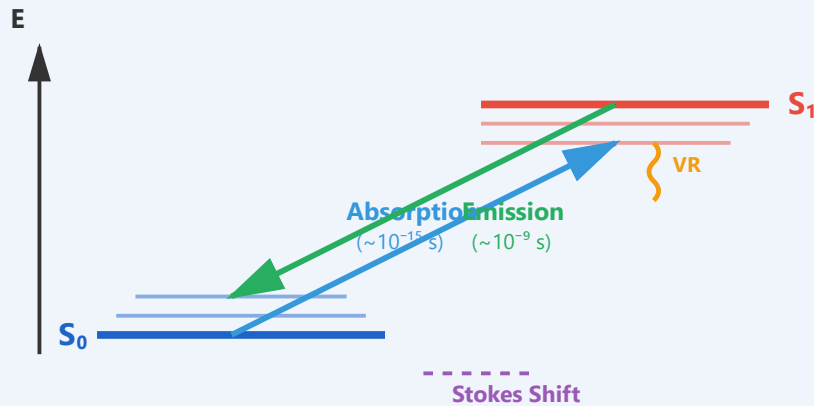


Fluorescence Principles

Jablonski Energy Diagram



Jablonski Diagram

$S_0 \rightarrow S_1$ (absorption)
 \downarrow vibrational relaxation
 $S_1 \rightarrow S_0$ (emission)

Timescales:

Absorption: $\sim 10^{-15}$ s
VR: $\sim 10^{-12}$ s
Emission: $\sim 10^{-9}$ s

Stokes Shift:

$\lambda_{\text{emission}} > \lambda_{\text{excitation}}$



Excitation/Emission Spectra

Mirror image relationship due to vibrational structure
Stokes shift separation enables detection
Peak wavelengths for filter optimization
Spectral overlap considerations for multicolor imaging



Fluorophore Properties

Brightness: $\epsilon \times \Phi$ (extinction \times quantum yield)
Lifetime: τ (1-10 ns typical)
Stokes shift: 20-100 nm
Photostability: varies widely between fluorophores



Photobleaching

Irreversible fluorescence loss over time
Reactive oxygen species (ROS) mediated damage
Antifade reagents help preserve signal
Limits long-term imaging duration



FRET Basics

Förster Resonance Energy Transfer

Distance-dependent (2-10 nm range)
Requires donor-acceptor pair
Molecular ruler for protein interactions