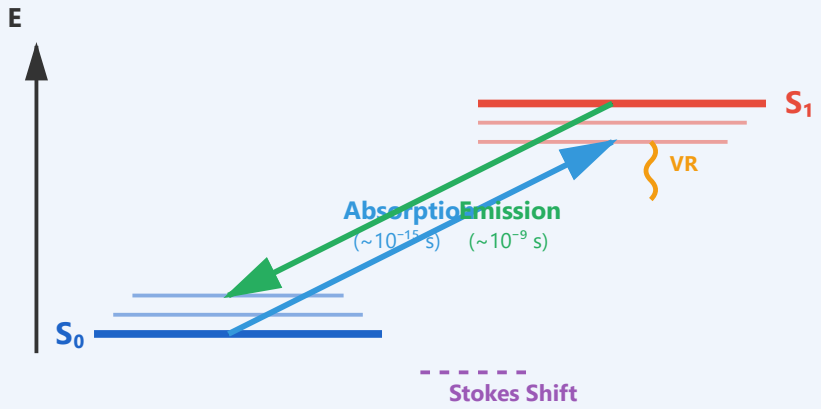
Fluorescence Principles

Jablonski Energy Diagram



Jablonski Diagram

$S_0 \rightarrow S_1$ (absorption)

↓ vibrational relaxation

$S_1 \rightarrow S_0$ (emission)

Timescales:

Absorption: ~10^{-15} s

VR: ~10^{-12} s

Emission: ~10^{-9} s

Stokes Shift:

λ_emission > λ_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: ε × Φ (extinction × 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