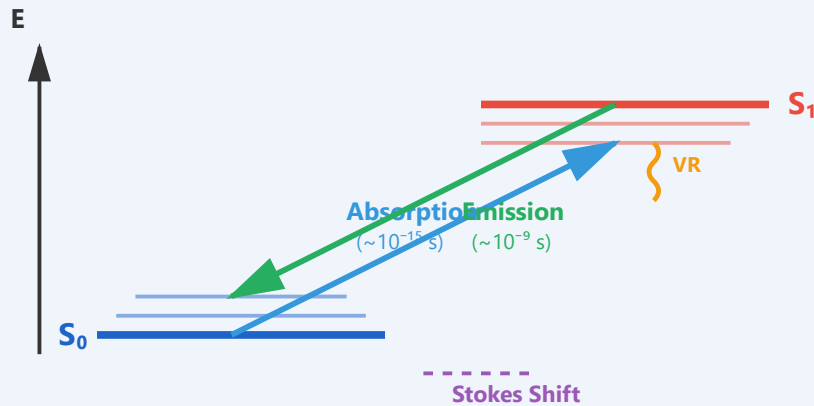


# Fluorescence Principles

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:**  $\tau$  (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