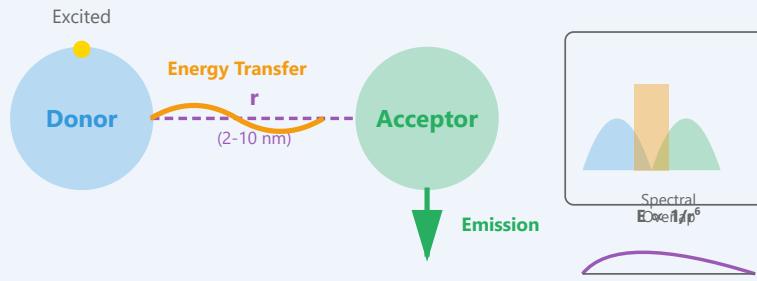


# FRET and Molecular Interactions

## Förster Resonance Energy Transfer Mechanism



## FRET Efficiency

$$E = R_0^6 / (R_0^6 + r^6)$$

$R_0$ : Förster radius

$r$ : Donor-acceptor distance

When  $r = R_0$ ,  $E = 50\%$

**Highly distance-dependent:**

$1/r^6$  relationship makes FRET exquisitely sensitive to small distance changes

## $R_0$ Calculations

Förster radius typically 2-10 nm  
Depends on spectral overlap  $J(\lambda)$   
Quantum yield of donor  
Orientation factor  $\kappa^2$

## FRET Pairs

**Classic:** CFP-YFP  
**Red-shifted:** GFP-RFP  
**Organic dyes:** Alexa Fluor, ATTO  
**Quantum dots:** Semiconductor nanocrystals

## Biosensor Design

Conformational change sensors  
**Examples:**  $\text{Ca}^{2+}$ , cAMP, kinases  
Protein-protein interactions  
Enzyme activity reporters

## Live Cell Applications

Real-time protein interactions  
Signaling pathway dynamics  
Molecular proximity measurements  
Drug screening assays