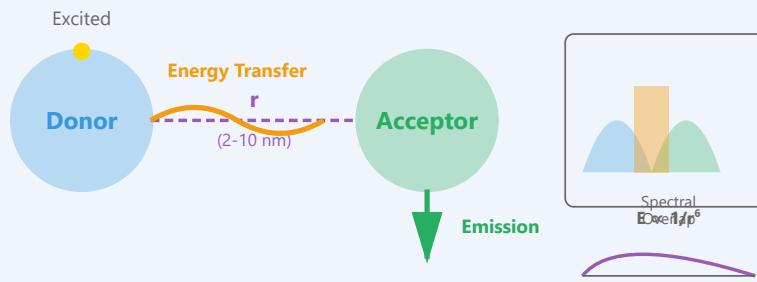


FRET and Molecular Interactions

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 κ^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: 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