Fluorescent signal transduction principles

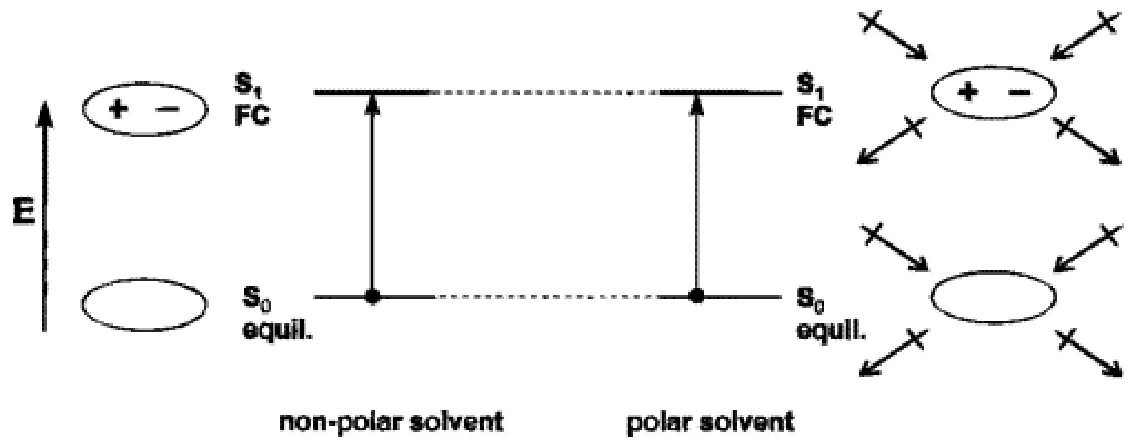
General issues of fluorescent detection

- Size of signaling unit (small organics vs. QDs)
- Molar absorptivity (ε) : how strongly a species absorbs light at a given wavelength
- Fluorescence quantum yield (Φ)
- Excitation wavelength (λ_{exc})
- Emission wavelength (λ_{em})
- Stokes shift
- Fluorescence lifetime
- Photostability (vs. photobleaching)
- Solubility

Signal transduction strategies

- Polarity probes
- Internal charge transfer (ICT) systems
- Photoinduced electron transfer (PET) systems
- Excimers
- Energy transfer systems

Effect of solvent polarity on absorbance



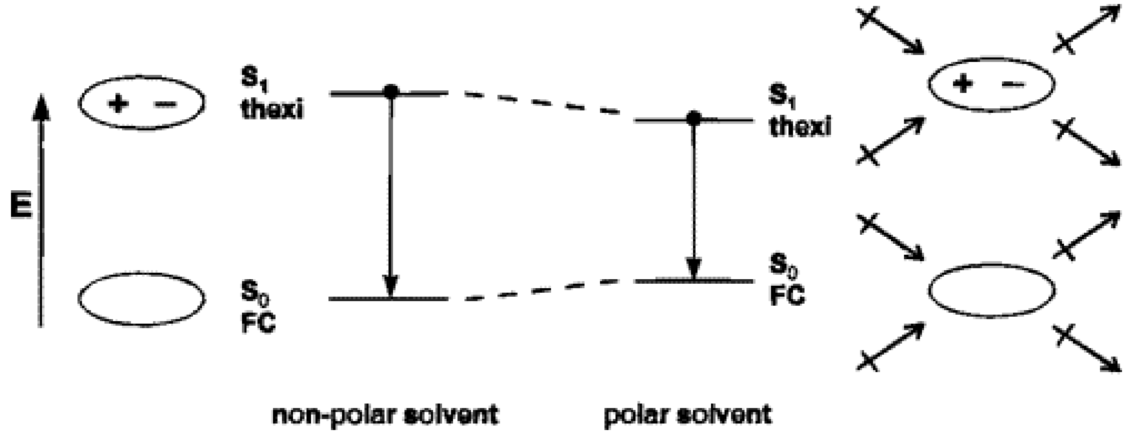
Absorption of energy / Excitation

Solvent shell arranges randomly

After excitation it is conserved in an unbiased manner

Little stabilization effect

Effect of solvent polarity on fluorescence



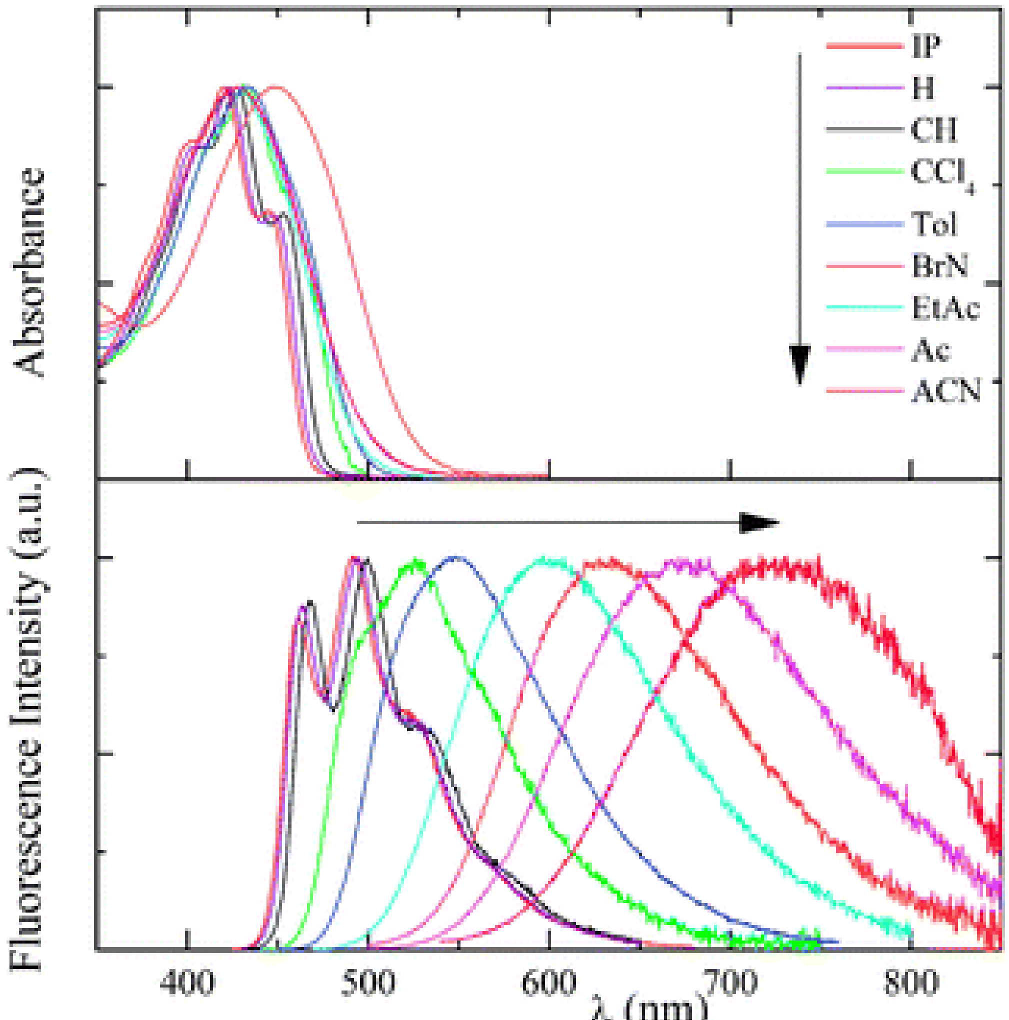
Relaxation / emission

Time scale is longer

Solvent relaxation stabilizes

Solvatochromism

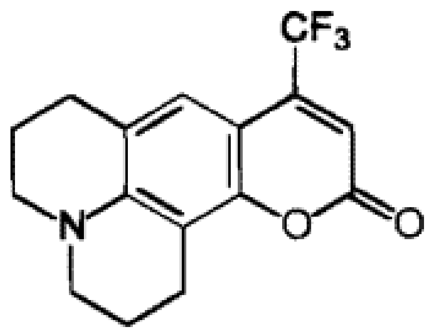
Effect of solvent polarity on absorbance and fluorescence

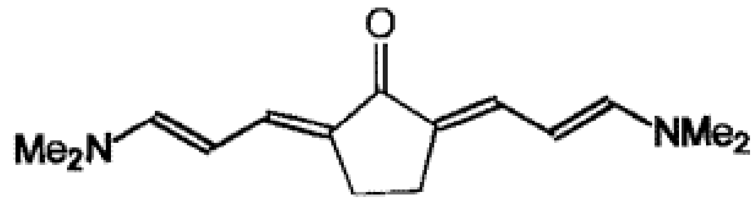


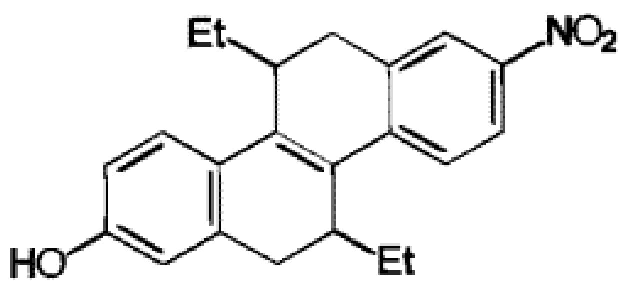
Polarity probes

- Change in dipole moment
- Electron donating and electron withdrawing groups (push-pull)
- Blue- or red-shifts
- In general the spectrum is red-shifted in polar solvents

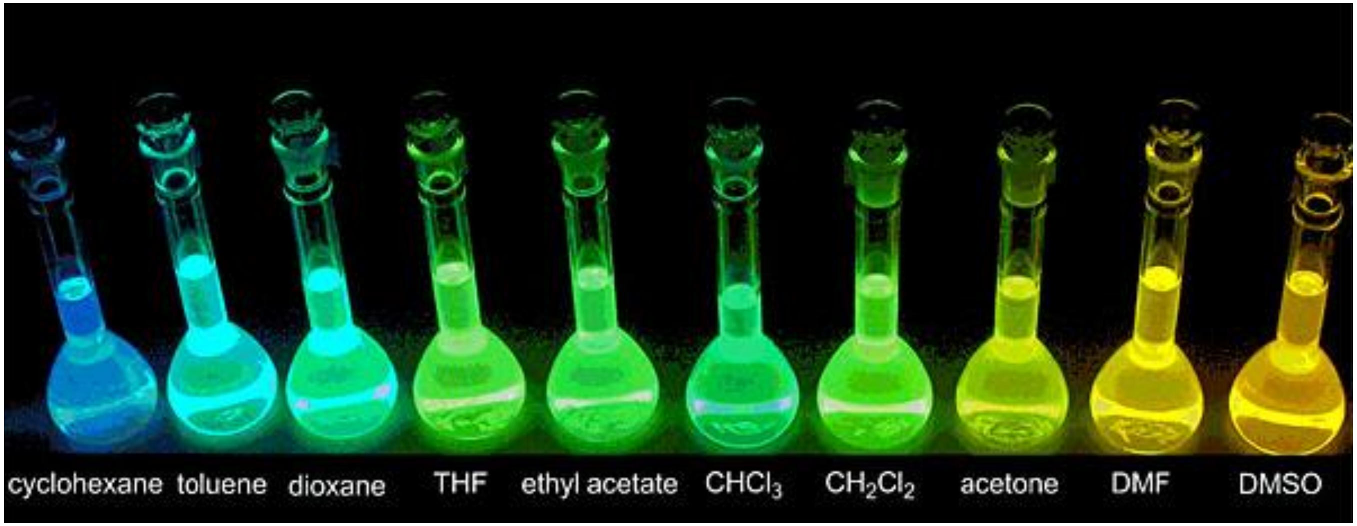
Signals





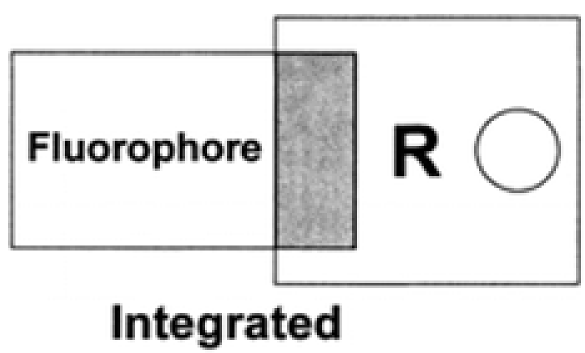


Signals

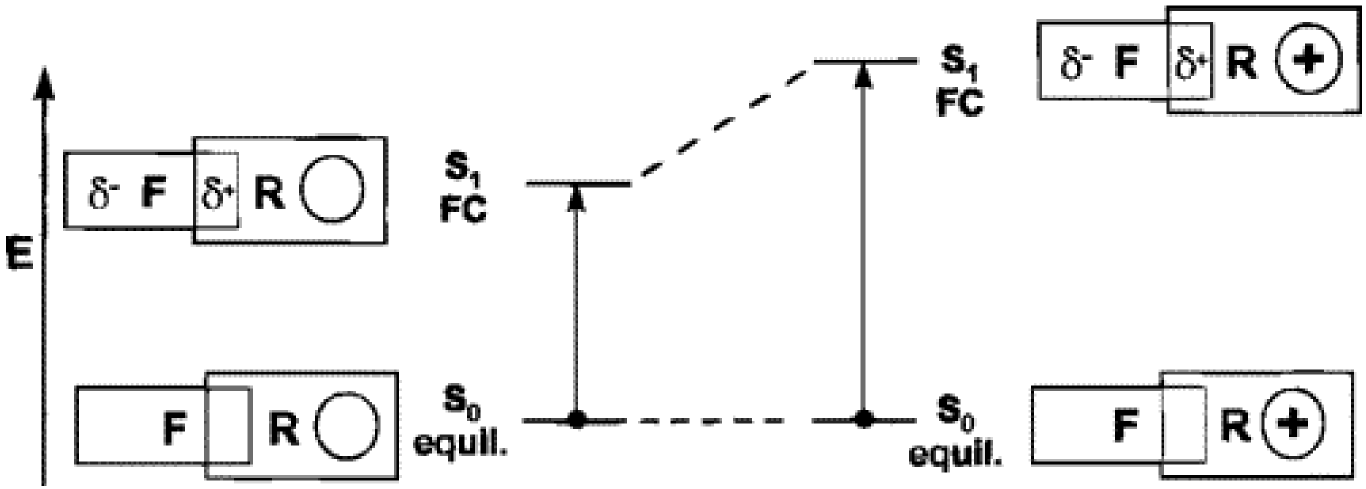

cyclohexane toluene dioxane THF ethyl acetate $CHCl_3$ CH_2Cl_2 acetone DMF DMSO

Internal charge transfer (ICT) sensors

- The primary excited state (locally excited) rearranges very fast to a charge separated species (large dipole
- Receptor and signaling units are integrated
- Principle is similar to solvatochromism, but more pronounced (definite charge is present)
- Charged guest will greatly influence the energy of the excited state (stabilize / destabilize)

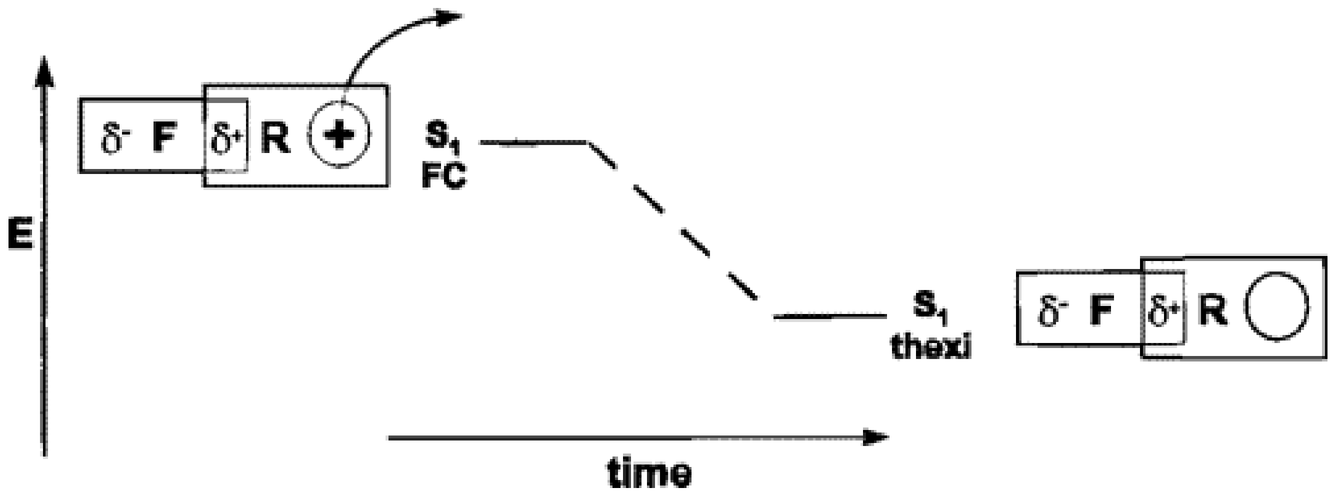


Mechanism of action in ICT sensors



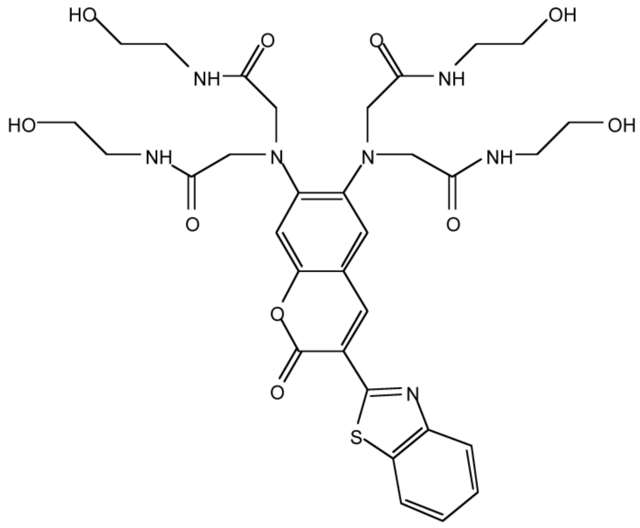
Both exitation and emission are affected (blue shifted here)

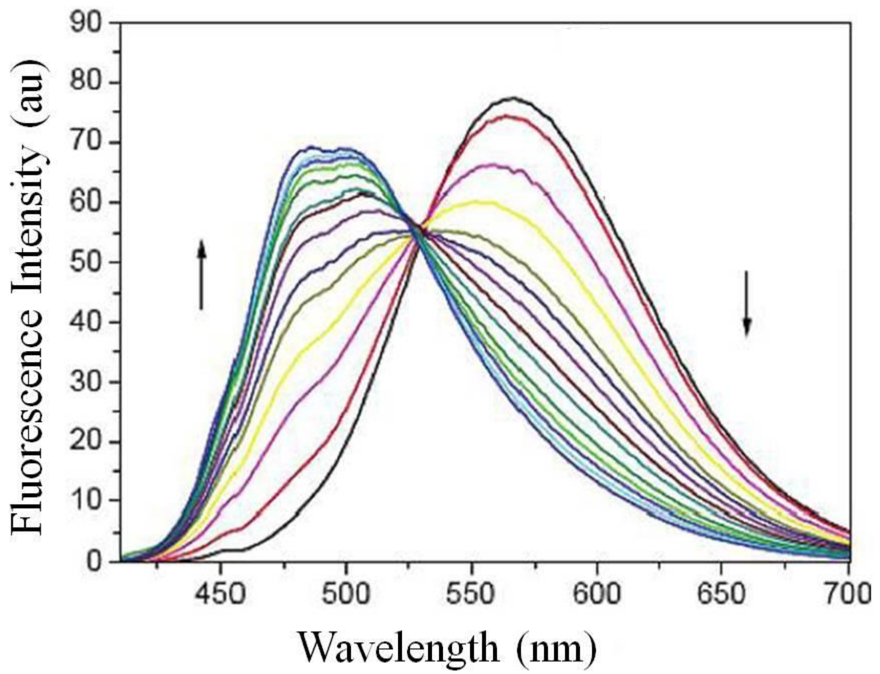
Dumpening of guests



- When repulsive interaction is present, the guest will be ejected
- K_a in the ground and excited states will be different
- K_a ground state is measured using absorption, Ka excited state from fluorescence

Examples

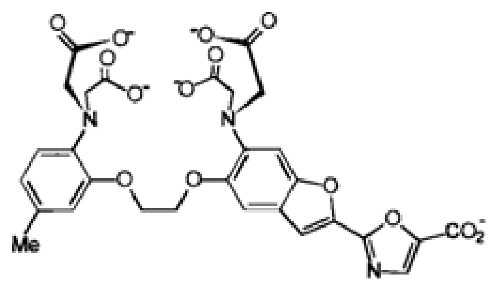




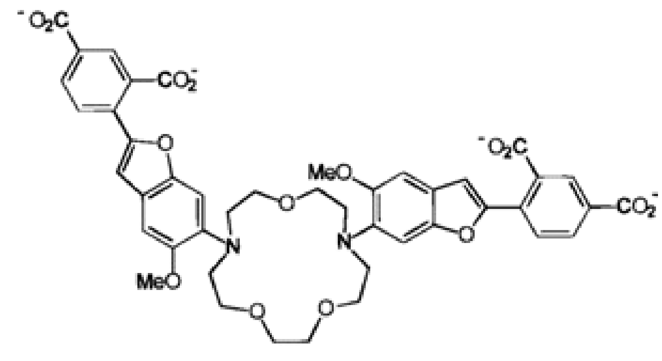
Fluorescence response in the presence of cations : control (0), Cd^{2+} (1), Hg^{2+} (2), Fe^{3+} (3), Zn^{2+} (4), Ag^{+} (5), Co^{2+} (6), Cu^{2+} (7), Ni^{2+} (8), and Pb^{2+} (9)

Fluorescence changes upon addition of Hg^{2+} (0 μM to 200 μM) in 0.05 M phosphate-buffered water solution (pH 7.5) with an excitation of 390 nm.

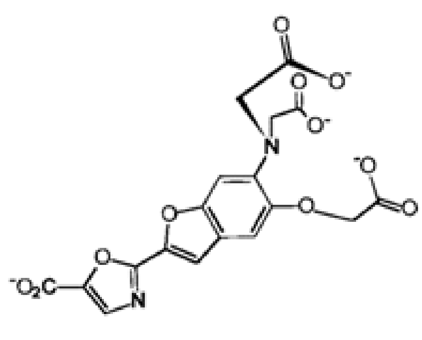
Examples



Ca^{2+}

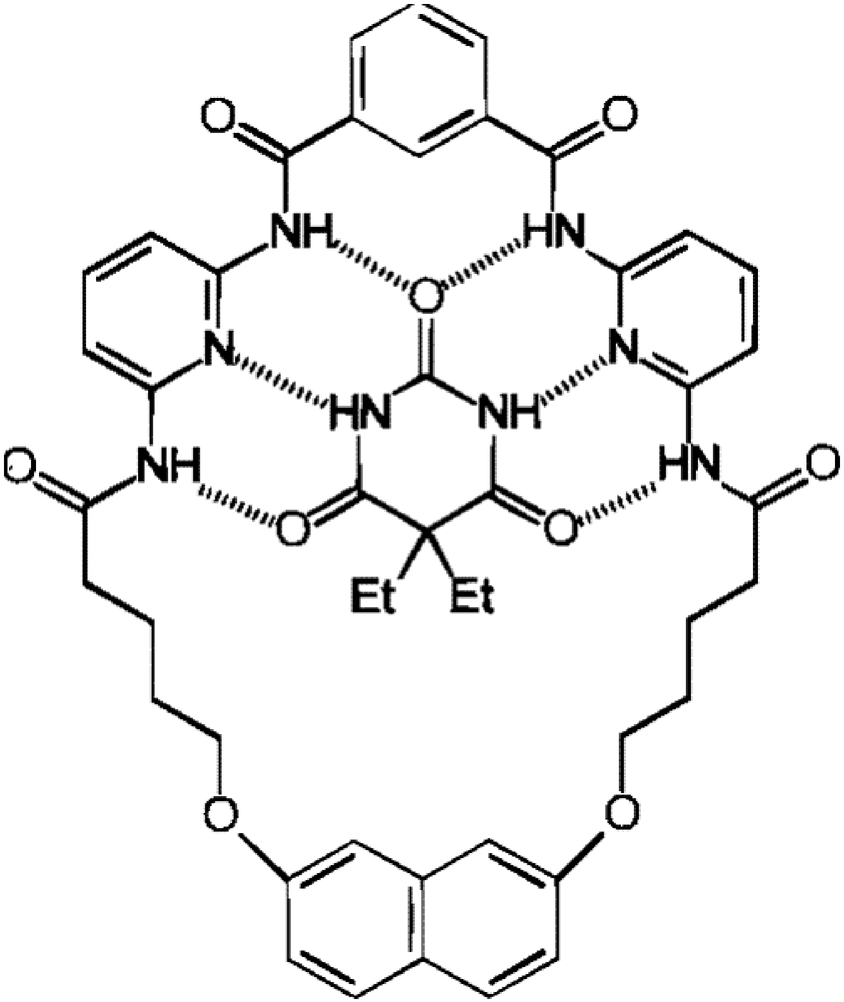


Na^{+}



Mg^{2+}

Detecting small organics with ICT



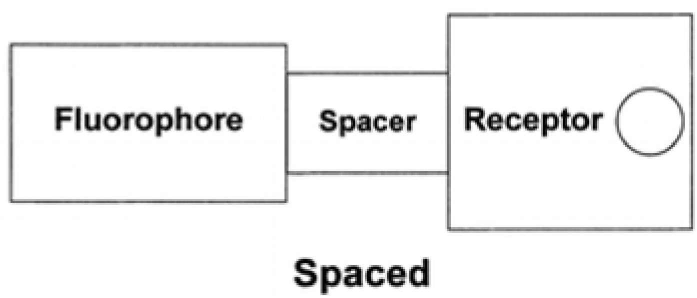
• acts via different transduction pathways

• no charge present

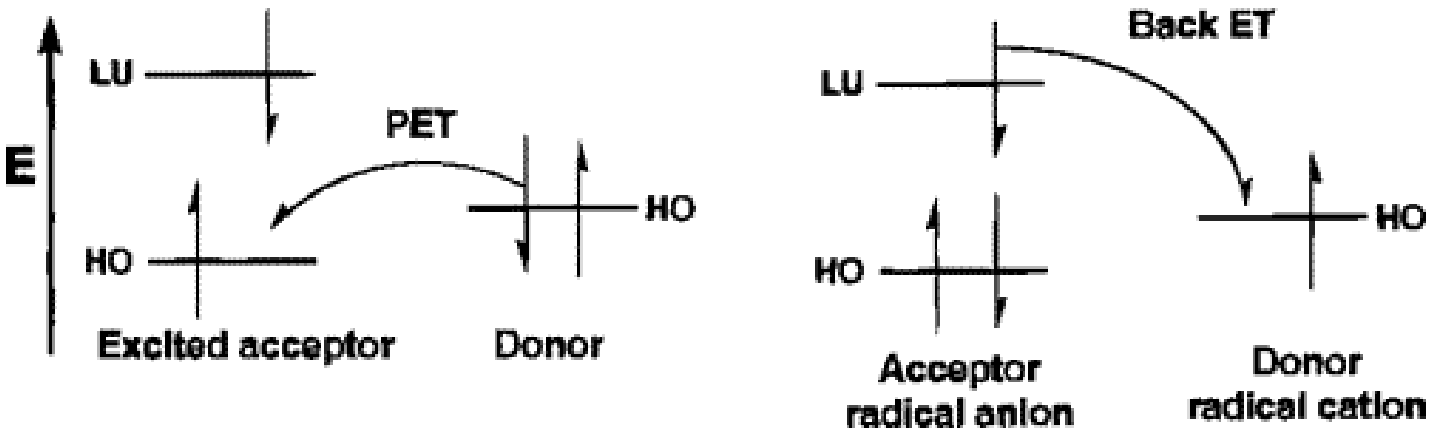
•Rigidifies the sensors, fluorescence increases (less vibrational / internal conversion relaxation)

Photoinduced electron transfer systems

- Receptor and signaling units are separated
- Receptor and fluorophore make up a redox pair
- Redox properties are influenced by guest binding



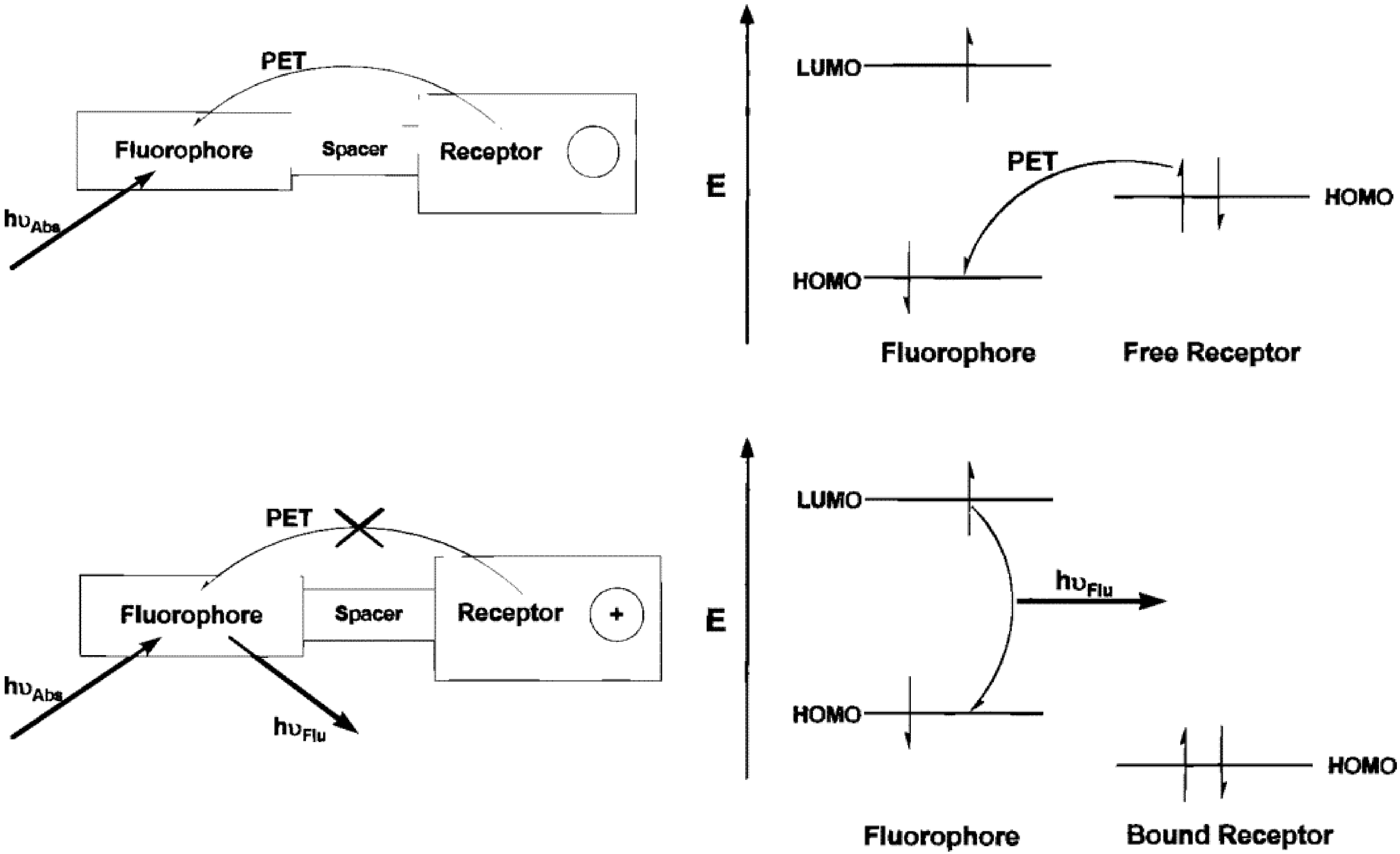
Mechanism of action of PET sensors



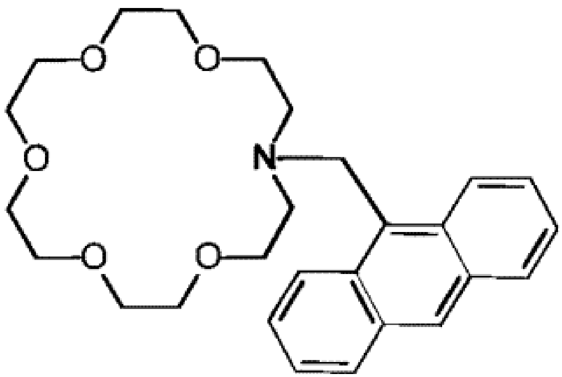
fluorophore receptor fluorophore receptor

- The electron donor in the receptor (reducing agent) reduces the excited state fluorophore
- fluorescence is quenched

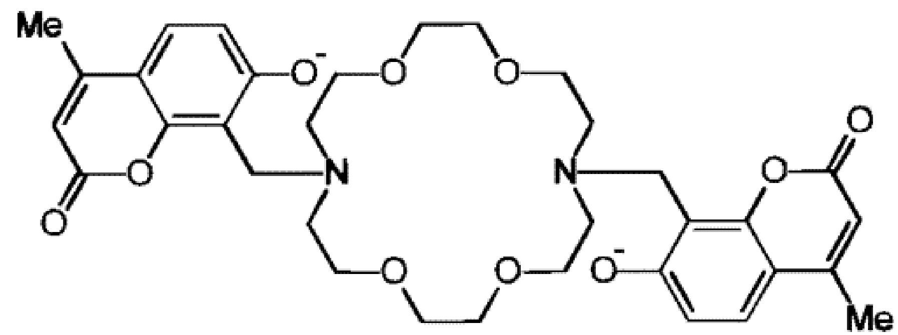
OFF-ON PET sensors



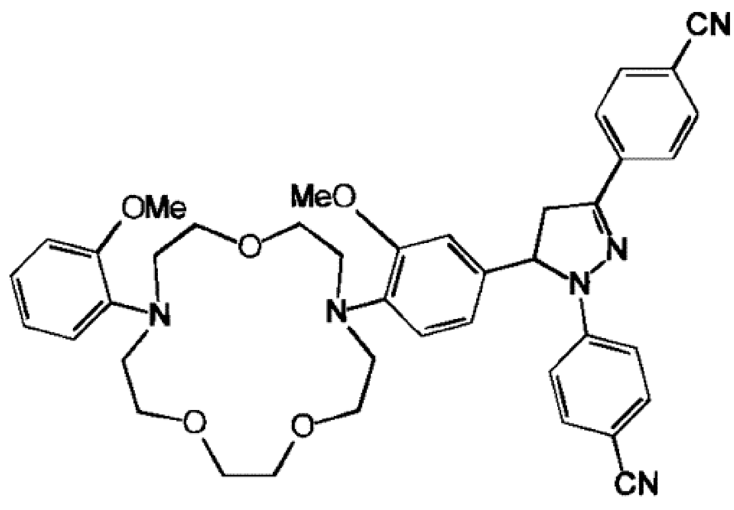
OFF-ON PET sensors



K^+

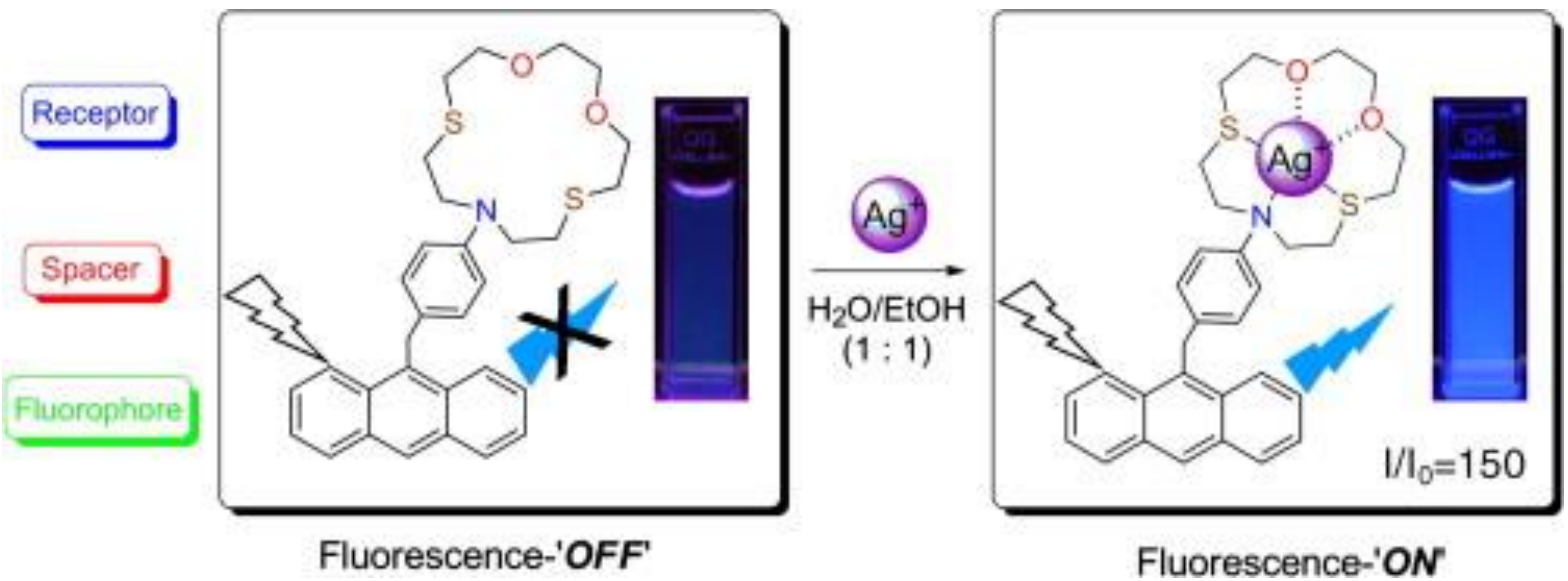


Ca^{2+}

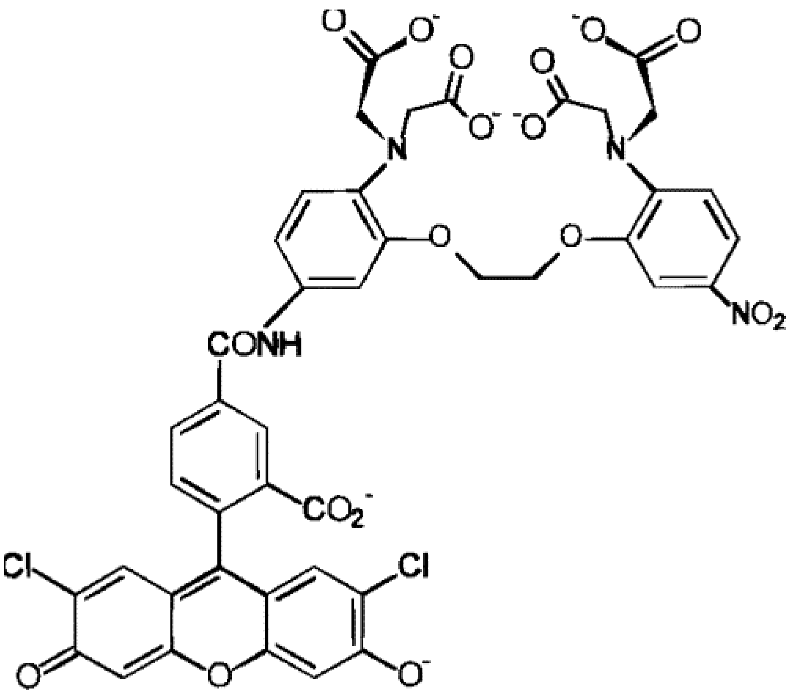


Na^+

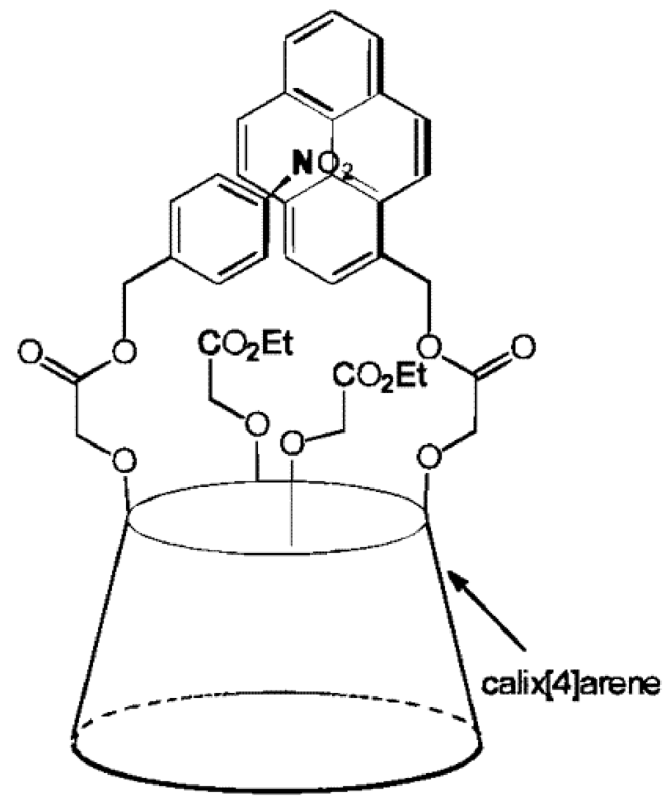
OFF-ON PET sensors



OFF-ON PET sensors

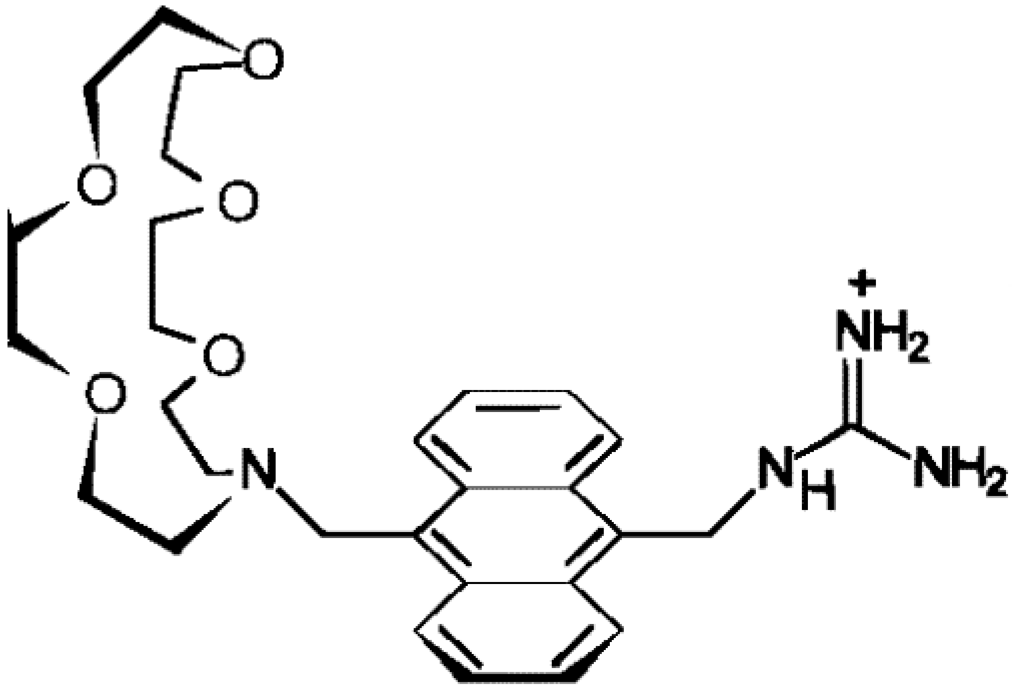


Ca^{2+}



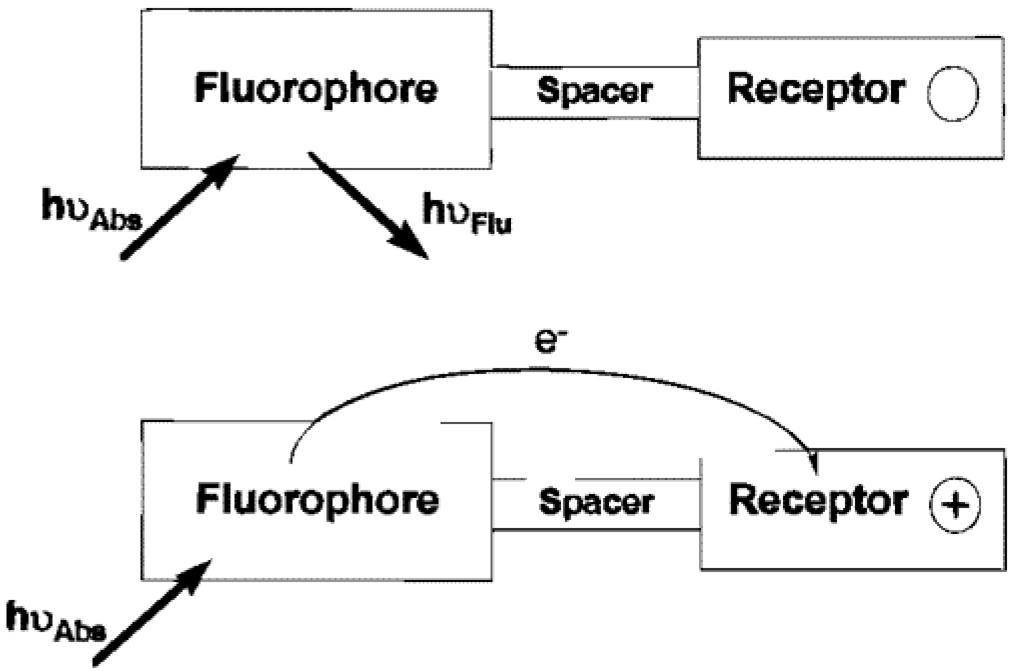
Na^{+}

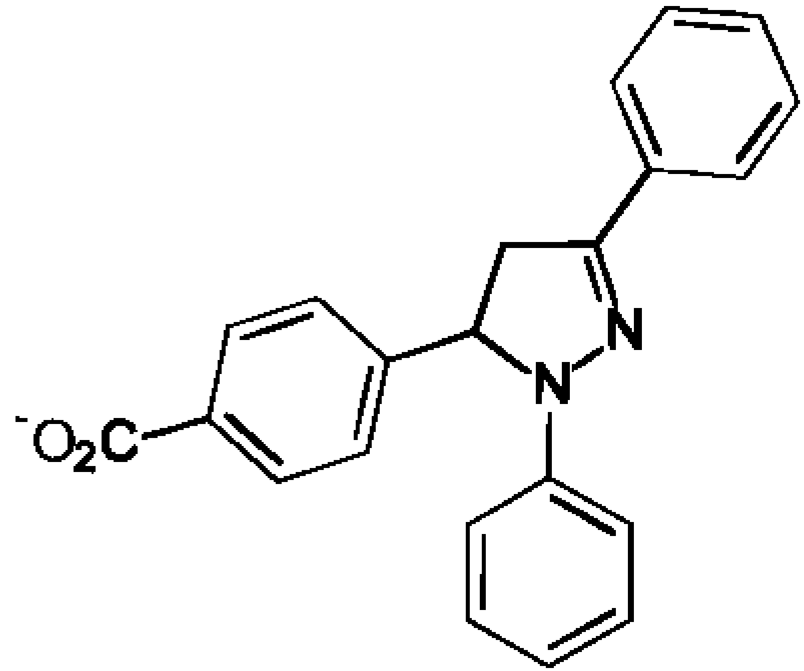
OFF-ON PET sensors



Guest = gamma aminobutiric acid (GABA)

ON-OFF PET sensors





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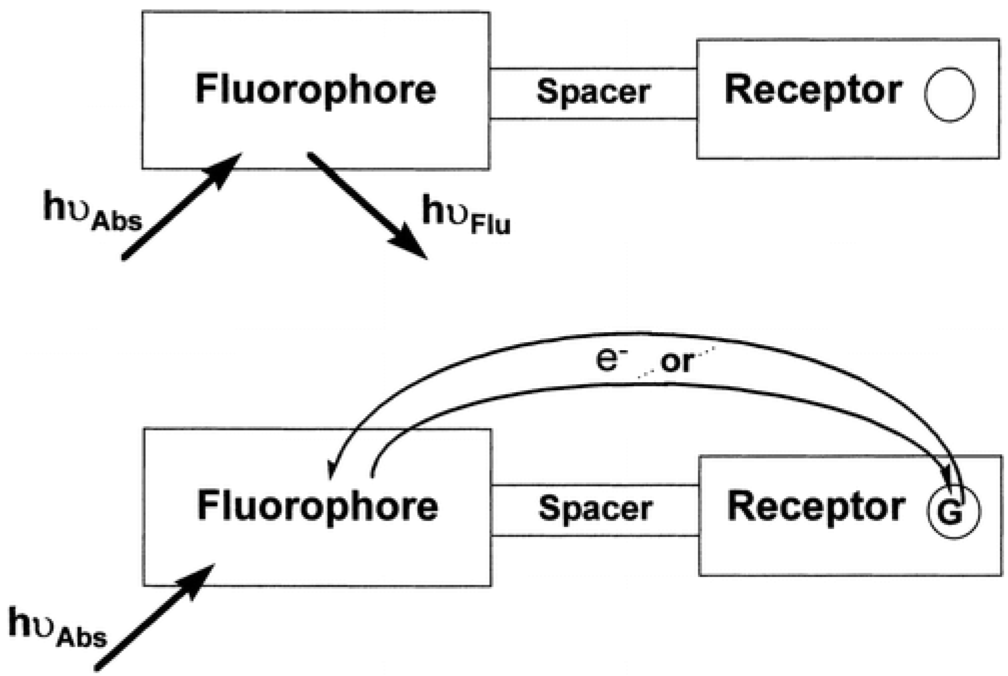
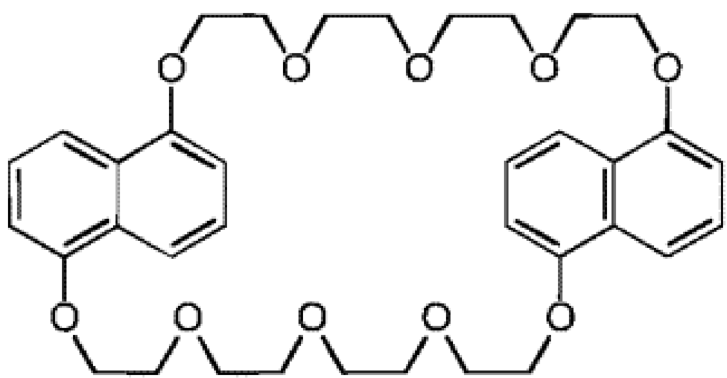
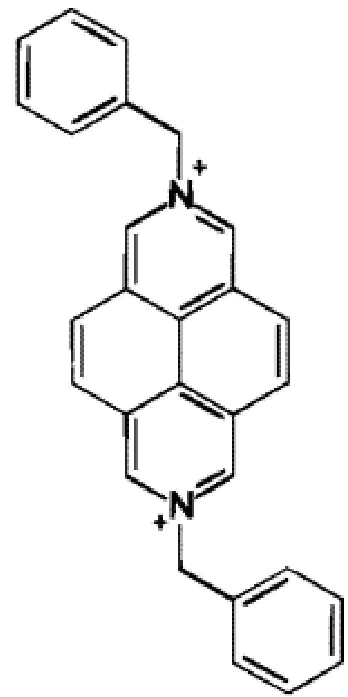
Redox active guests in PET sensors



Redox active guests in PET sensors



PET sensor

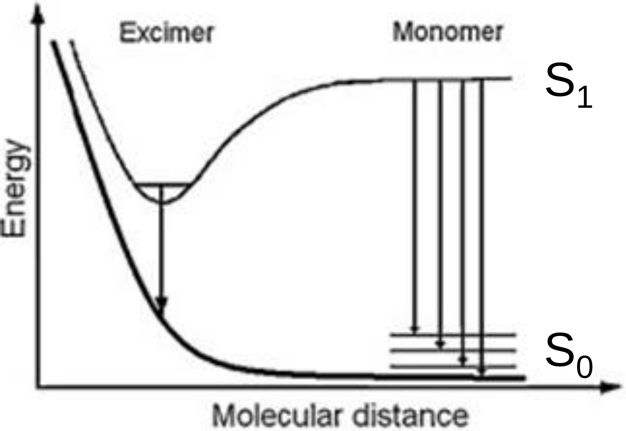


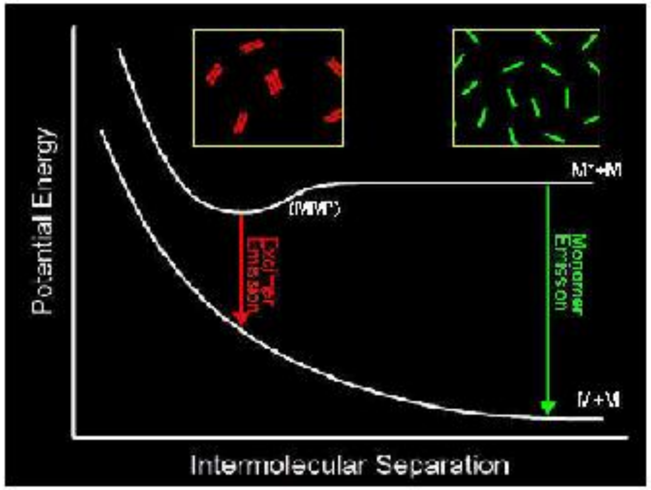
Redox active guest

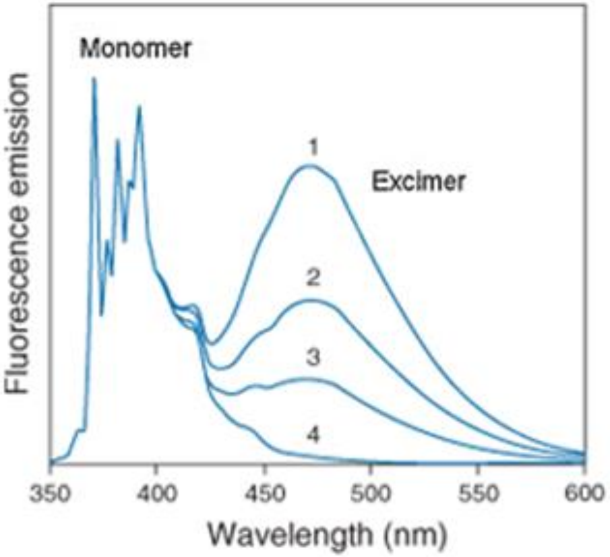
Monomer-excimer systems

- Two or more fluorophores are needed
- F + F* → [FF]*
- Excited dimer
- Diffusion controlled
- Homodimer (excimer), heterodimer (exciplex)
- Mainly hydrocarbon fluorophores (pyrene, anthracene etc.)

Energetics of excimer formation





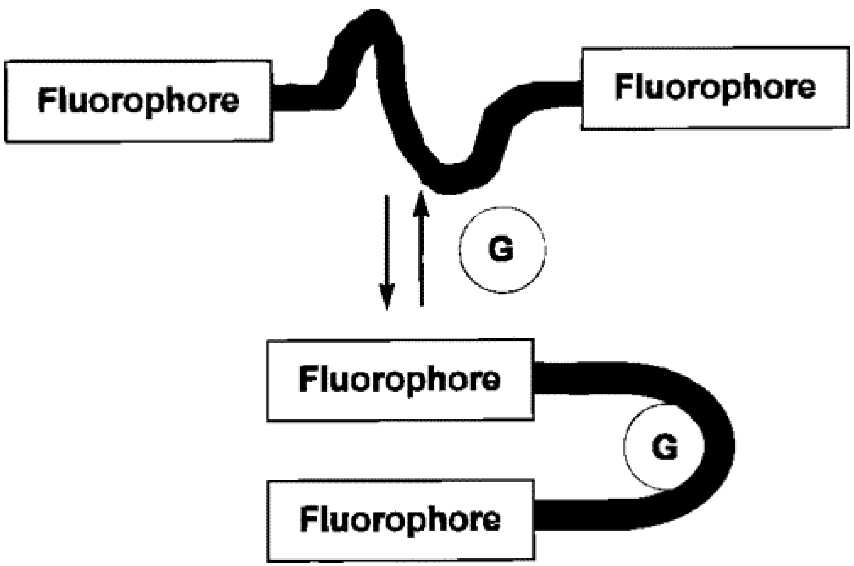


- excimer band is always structureless and red shifted
- Monomer / excimer ratio is independent of concentration
- two-point-calibration

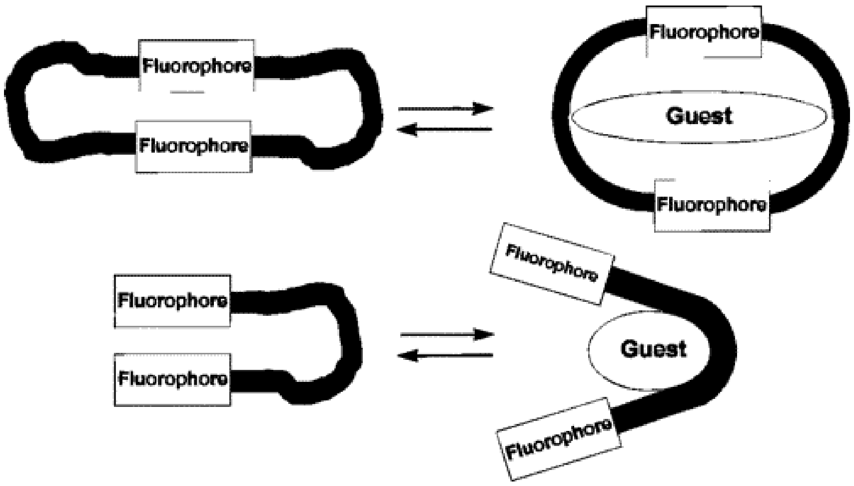
Excimer sensors

- In sensors: guest induced formation or dissociation

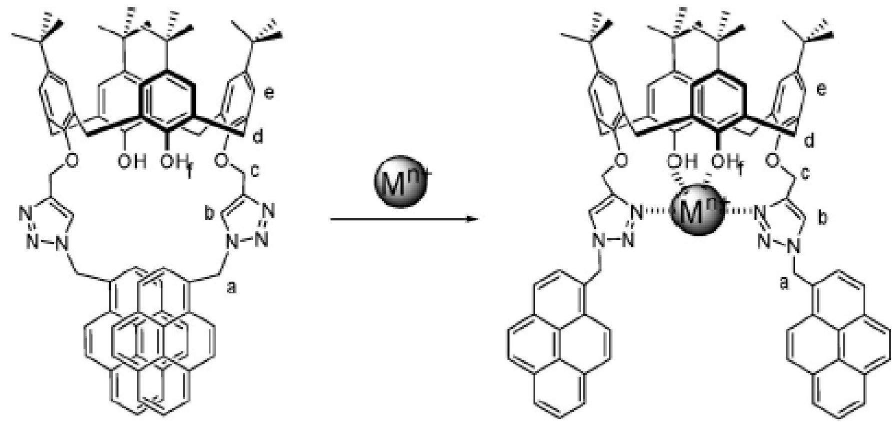
Guest induced formation



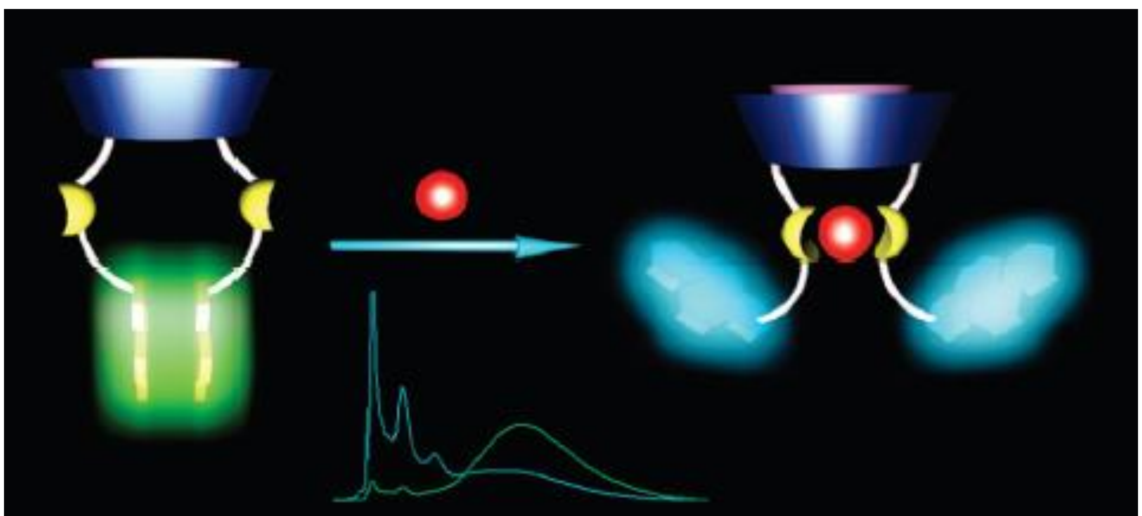
Guest induced dissociation



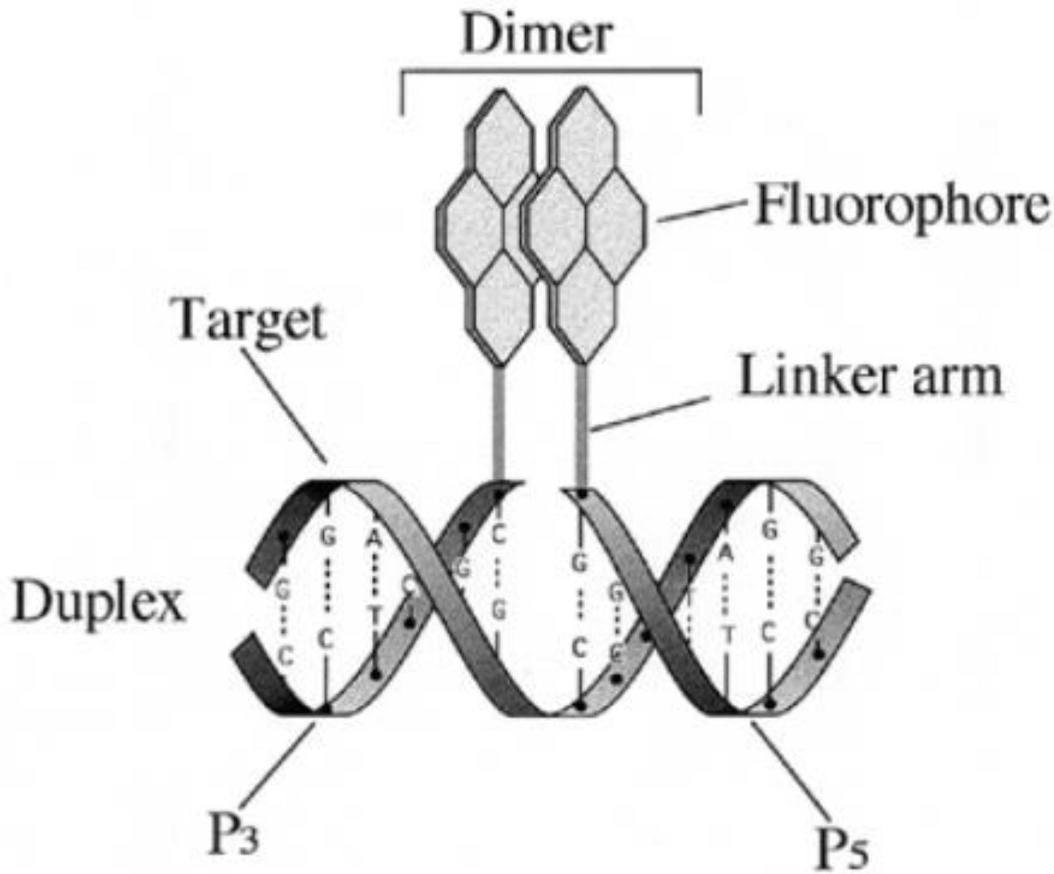
Examples



= Cd^{2+}

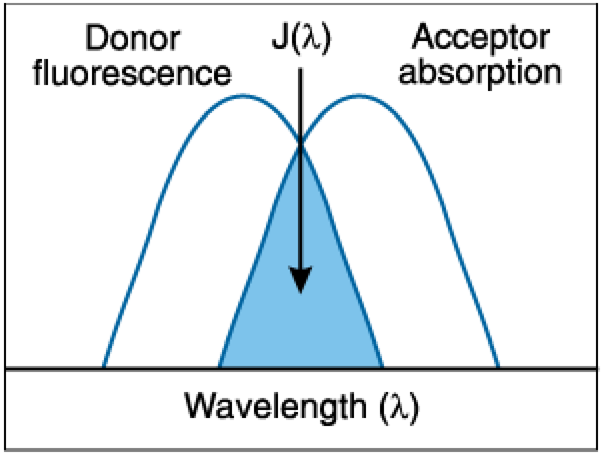


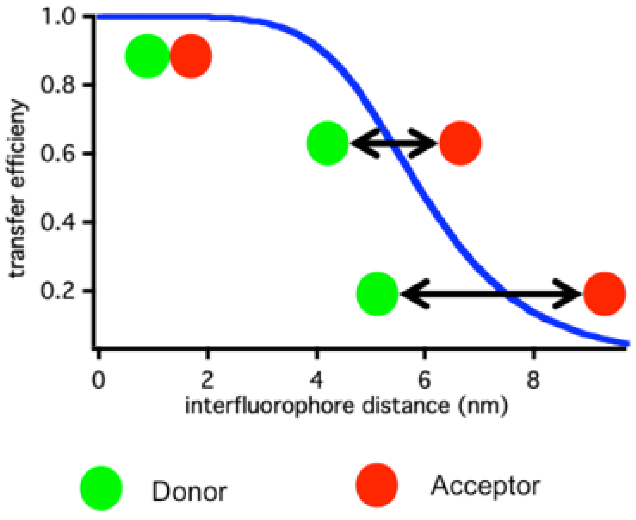
Examples



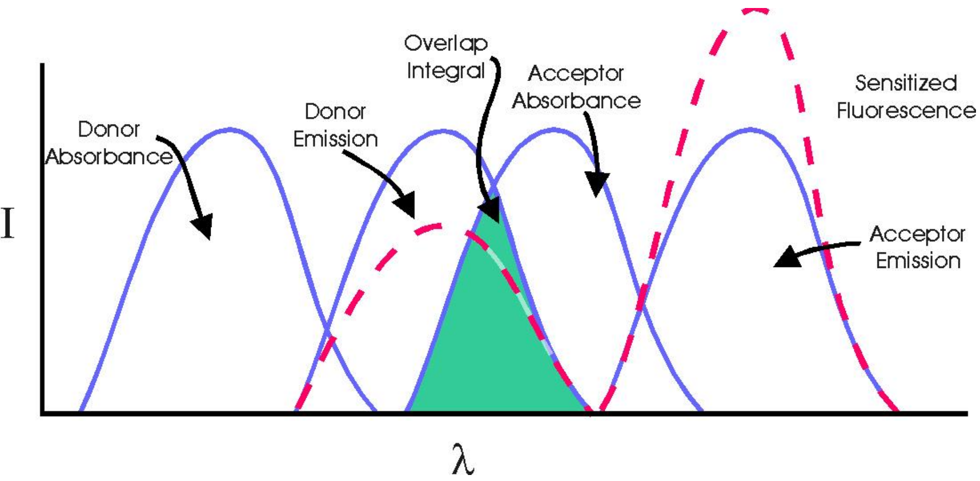
Energy transfer systems

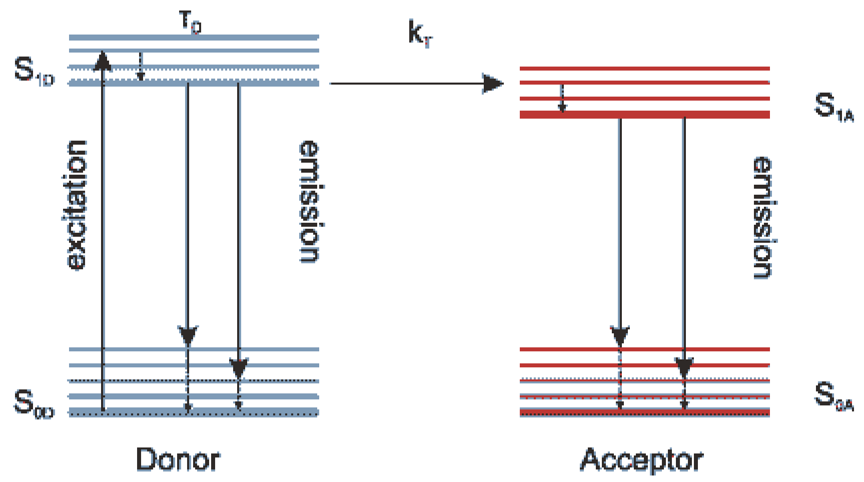
- Two or more fluorophores are needed
- A + D* → A* + D (vs. Inner filter effect)
- Spectral overlap, transition moments
- Distance (<100 Å)





Energy transfer systems





- Short range (Dexter) mechanism : electron exchange between overlapping molecular orbitals (<10 Å)

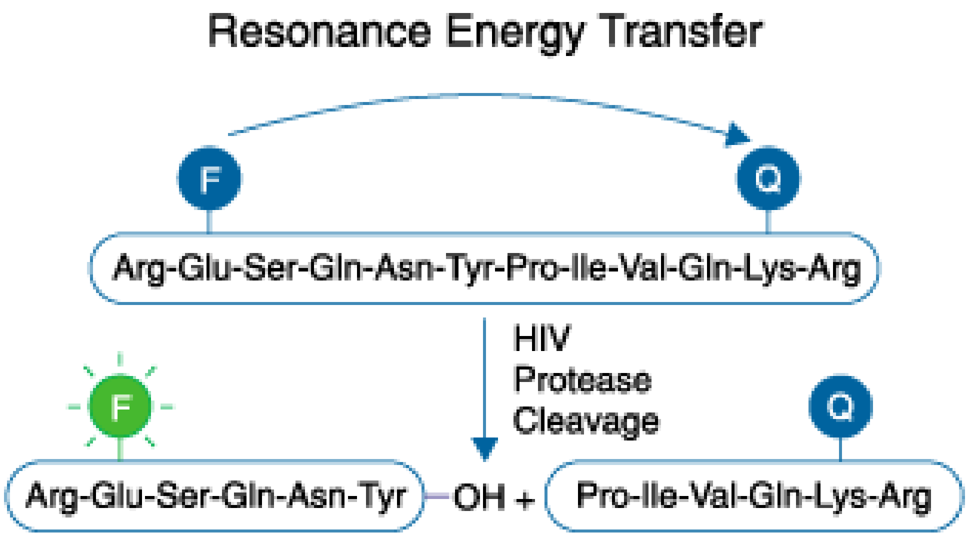
- Long range (Förster) mechanism : coulombic interactions between opposed dipole moments

- ET depends on $E = \dfrac{1}{1 + (r/R_0)^6}$

Energy transfer systems

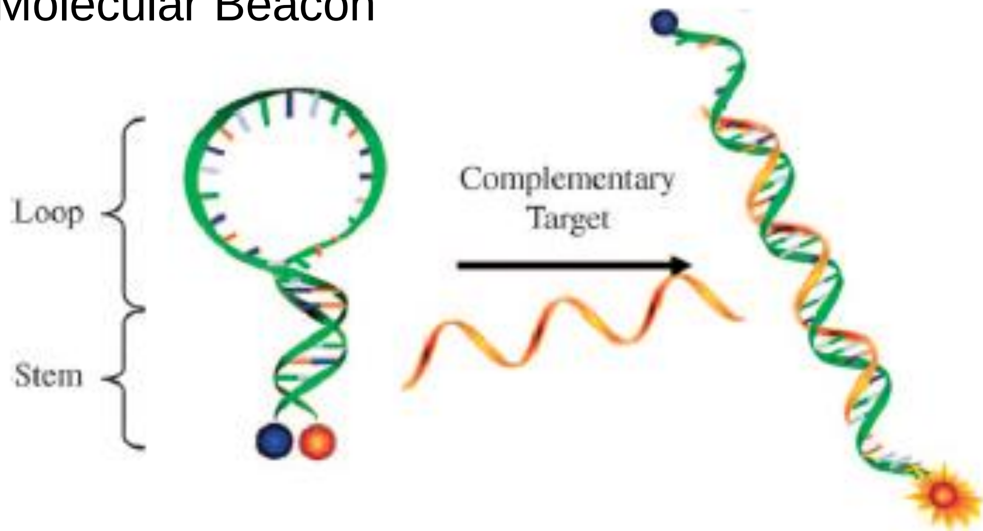
- Förster type of energy transfer (FRET)
- Strongly distance dependent (molecular ruler)
- Enzyme kinetic measurements
- HOST-GUEST (receptor-ligandum)
- Membrane diffusion / fusion
- Conformational changes
- Colocalisation
- Imaging techniques (resolution increase)

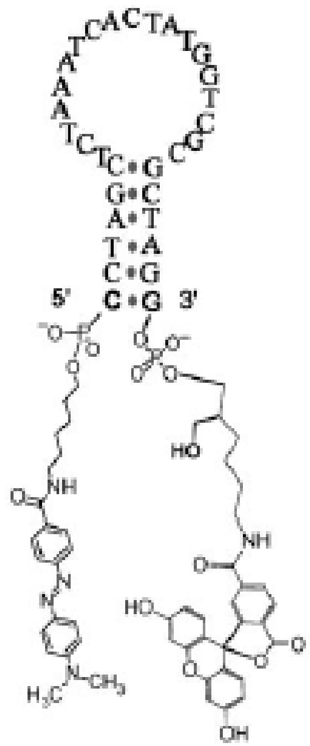
Examples

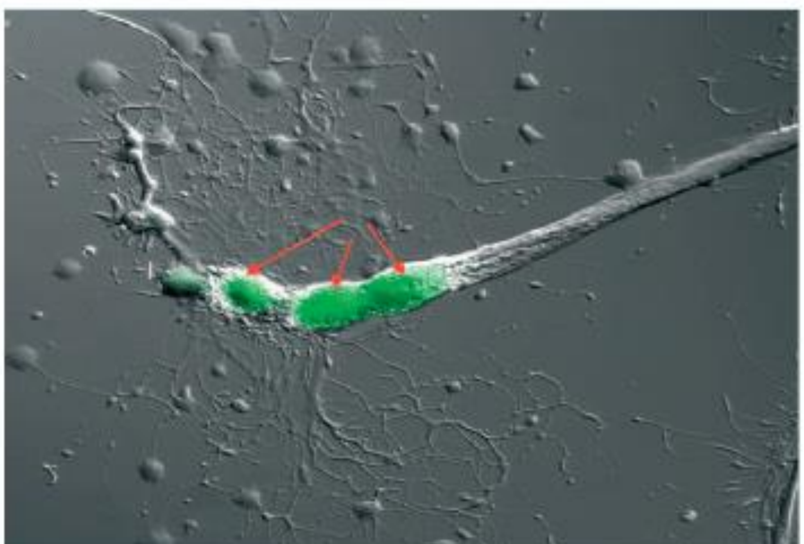

Resonance Energy Transfer
F
Q
Arg-Glu-Ser-Gln-Asn-Tyr-Pro-Ile-Val-Gln-Lys-Arg
HIV
Protease
Cleavage
F
Q
Arg-Glu-Ser-Gln-Asn-Tyr
OH +
Pro-Ile-Val-Gln-Lys-Arg

Examples

Molecular Beacon







Examples

GFP-based probes