Electron-Exchange Quenching

Figure 1 shows a schematic for the electron exchange or Dexter interaction. This interaction occurs between a donor *D*E and an acceptor *A*E, where *E* indicates electron exchange. The excited donor has an electron in the LU orbital. This electron is transfer to the acceptor. The acceptor then transfers an electron back to the donor. This electron comes from the HO orbital of the acceptor, so the acceptor is left in an excited state. Electron exchange is similar to RET because energy is transferred to an acceptor. This mode of energy transfer also depends on spectral overlap of the donor and acceptor, just like RET. In contrast to RET, the Dexter interaction is a quantum mechanical effect that does not have an analogy in classical electrodynamics. RET is well known to result in an excited acceptor. In contrast, the Dexter interaction is usually associated with quenching. This association occurs because RET occurs over large distances, so if there is spectral overlap the transfer occurs by RET before Dexter transfer can occur. Dexter transfer may occur at short donor–acceptor distances, but the donor will be completely quenched by RET or Dexter transfer, and thus non-observable. Dexter transfer can be observed if the spectral overlap is small, so that the large rates of exchange become significant. Additionally, high concentrations are needed for significant Dexter transfer, whereas RET occurs at much lower concentrations. For an unlinked donor and acceptor the bulk concentration of the acceptor needs to be about 10^{-2} M to have an average distance of 30 Å. For a fluorophore and quencher the bulk quencher

concentration needs to be near 1 M to obtain an average distance of 6.5 Å.

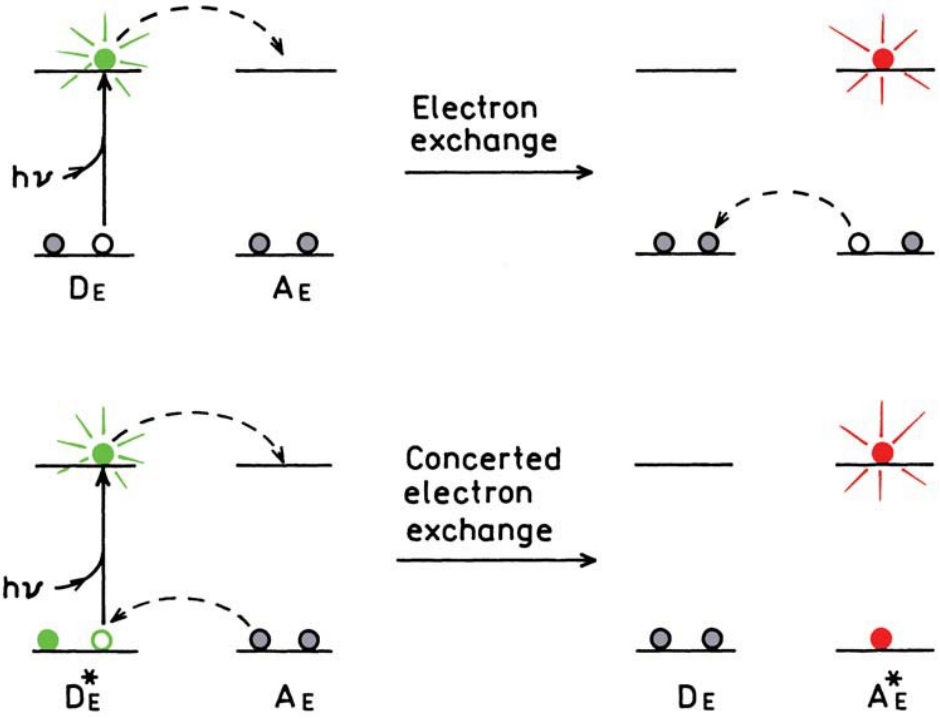


Figure 1. Schematic for stepwise (top) or concerted (bottom) electron exchange.

Photo induced Electron Transfer

The third mechanism for quenching is photo induced electron transfer (PET). In PET a complex is formed between the electron donor *D*P and the electron acceptor *A*P, yielding $D_P^+A_P^-$ (Figure 2). The subscript P is used to identify the quenching as due to a PET mechanism. This charge transfer complex can return to the ground state without emission of a photon, but in some cases exciplex emission is observed. Finally, the extra electron on the acceptor is returned to the electron donor. The terminology for PET can be confusing because the excited fluorophore can be

either the electron donor or acceptor. The direction of electron transfer in the excited state is determined by the oxidation and reduction potential of the ground and excited states. When discussing PET the term donor refers to the species that donates an electron to an acceptor. In PET the terms donor and acceptor do not identify which species is initially in the excited state. This is different from RET, where a fluorophore is always the donor. The nature of PET quenching is clarified by examining several examples. The more common situation is when the excited state of a fluorophore acts as an electron acceptor . One typical example is an electron-rich species such as dimethylaniline (DMA), which can donate electrons to a wide range of polynuclear aromatic hydrocarbons, which act as electron acceptors. Electron transfer is even more favorable to electron-deficient species like cyanonaphthalenes. There are some unusual PET pairs, such as indole donating to pyrene and dienes donating to cyano anthracene. PET quenching can also occur by electron transfer from the excited fluorophore to the quencher. Examples include electron transfer from excited indoles to electron-deficient imidazolium or acrylamide quenchers. Quenching by halocarbons can also occur by electron transfer from the fluorophore to the electronegative halocarbon. Electron-rich dimethoxy naphthalene can donate electrons to pyridinium.

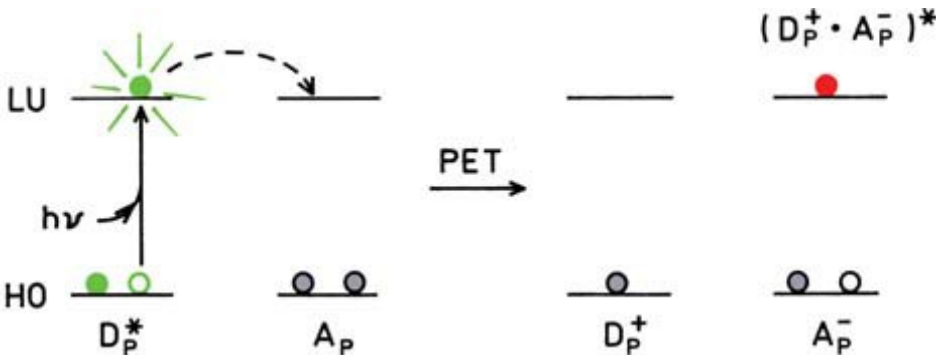


Figure 2. Molecular orbital schematic for photo induced electron transfer.

Fluorophore Dimerization and Isosbestic Points

Usually, dye aggregates are classified on the basis of the observed spectral shift of the absorption maximum relative to the respective absorption maximum of the monomer. For the majority of possible dimer geometries, two absorption bands arise, one at higher energy relative to the monomer band, termed H-type aggregates (absorption band shifted hypsochromic), and at lower energy relative to the monomer band, termed J-type or Scheibe-type aggregates (absorption band shifted bathochromic). J-type aggregates exhibit a bent or head-to-tail structure and usually show fluorescence with an intensity that fairly often surpasses that of the monomeric dyes. In contrast, it is known that the fluorescence of face-to-face-stacked H-type dimer aggregates (sandwich-type dimers) is strongly quenched. In fact, with the exception of a few examples , the non-emissive character of the excited state has become commonly accepted as a general feature of H-aggregates. According to exciton theory of Kasha et al. , in J-aggregates, only transitions to the low energy states of the

exciton band are allowed and, as a consequence, J-aggregates are characterized by a high fluorescence quantum yield. In contrast, H-aggregates are characterized by a large Stokes-shifted fluorescence that has a low quantum yield (Figure 3). After exciting the H-exciton band, a rapid downwards energy relaxation occurs to the lower exciton states that exhibit small transition dipole moments. Therefore, their fluorescence is suppressed and a low fluorescence yield characterizes the H-aggregates. These two different types of aggregates, the J- and H aggregates, are distinguished by the different angle a between the molecular transition dipole moments and the long aggregate axis. When a>54.7°, H-aggregates are formed and when a<54.7°, J-aggregates are formed. In general, when there is interaction between two or more molecules in the unit cell of the aggregate, two or more excitonic transitions with high transition moment are observed and the original absorption band is split into two or more components. This splitting depends on the distance between the molecules, the angle of their transition dipole moments with the aggregate axis, the angle of the transition dipole moments between neighboring molecules and the number of interacting molecules. The appearance of isosbestic points in the absorption spectrum with increasing dye concentration provides good evidence for an equilibrium between monomeric and dimeric species and enables the association constant to be calculated, in addition to the spectra of the pure monomer and dimer. A wavelength at which two or more components have the same extinction

coefficient is known as an isosbestic wavelength or isosbestic point.

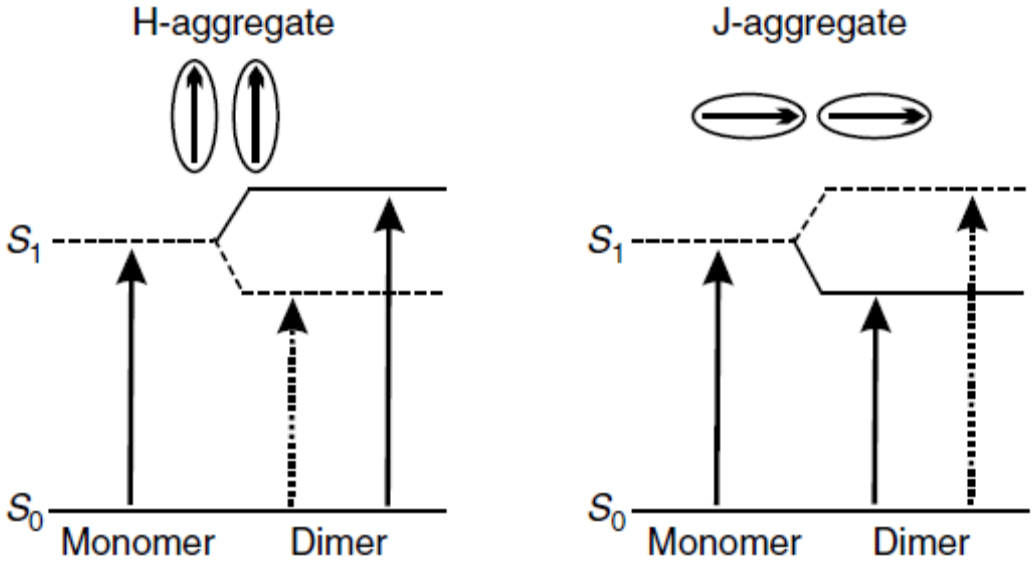


Figure 3. Simplified schematic of exciton theory to explain the different absorption and fluorescence behaviors of H- and J-aggregtes.

Stokes Shift, Solvent Relaxation, and Solvatochroism

As organic dyes consist of many atoms (typically 50–100) they thus show a manifold and complex vibrational spectrum. Accordingly, the fluorophore has a large number of energetically different transition possibilities to the vibrational ground state after excitation with light of appropriate wavelength. Owing to the solvation shell and corresponding interactions between fluorophores and solvent molecules, the resulting vibrational transitions are considerably broadened at room temperature. The complete shift of the fluorescence emission band compared with the absorption band, due to the radiationless deactivation processes, is called the Stokes Shift.

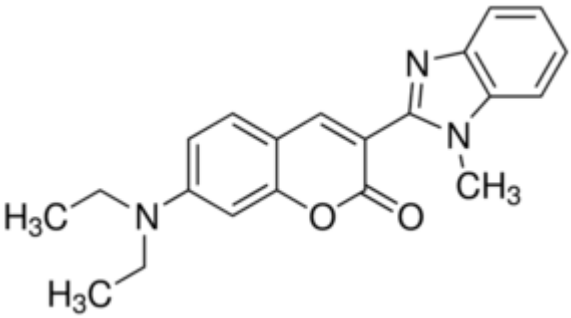
Because the electron distribution changes upon excitation, different bonding forces and dipole moments arise. Therefore, the solvent molecules experience a new equilibrium configuration, which they adjust to within several picoseconds at room temperature. The kinetics of dielectric relaxation of solvent molecules can be followed by monitoring the time-dependent shift in the fluorescence emission spectrum with picosecond time-resolution . If a fluorescing molecule is excited into a more polar excited state, the electronic polarization of the solvent molecules adjusts instantaneously to the new electron distribution in the molecule. In contrast, the orientational polarization of the solvent molecules does not change instantaneously with the excitation. Therefore, the orientational polarization is not in equilibrium with the excited molecule. This means that the solvent molecules have to react by dielectric relaxation until the equilibrium configuration of the corresponding excited state is reached.

Furthermore, owing to the different properties of the ground and excited states, the dipole moment changes upon excitation $\Delta\mu=\mu_e-\mu_g$, which is reflected in a shift in the absorption and emission band, which is dependent on solvent polarity (Solvatochromism). Therefore, charge transfer transitions, for example, in coumarin dyes, show pronounced solvatochromism effects. On the other hand, distinct shifts in absorption and emission of suitable candidates can be used advantageously for the definition of new solvent polarity parameters . For a complete description of solvatochromic effects, the refractive index n and the dielectric constant Ɛs of the solvent, in addition

to the change in dipole moment of the fluorophore upon excitation, $\Delta\mu$, have to be considered. Using the Lippert equation.

$$\nu_{abs}-\nu_{em}=\frac{2\left(\mu_e-\mu_g\right)^2}{chc a^3}\left[\frac{2(\varepsilon_s-1)}{(2\varepsilon_s+1)}-\frac{2(n^2-1)}{(2n^2+1)}\right]$$

the Stokes shift (n_{abs}_n_{em}) can be expressed as a function of solvent properties (n, Ɛs) and the dipole moments of the fluorophore in the ground, μ_g, and excited states, μ_e, where



Coum

arin dye

c is the speed of light, h is Planck's constant, and a the Onsager radius of the fluorophore in the respective solvent. While for coumarin dyes the Stokes shift generally increases with solvent polarity (i.e., the emission maximum shifts further bathochromically than the absorption maximum), rhodamine derivatives show negligible solvatochromism.

SEMICONDUCTOR NANOPARTICLES

Starting in 1998 there has been rapid development of fluorescent semiconductors nanoparticles. The main component of these particles is usually cadmium selenide (CdSe), but other semiconductors are also used. Particles of

CdS, CdSe, InP, and InAs (Indium arsenide) with diameters ranging from 3 to 6 nm can display intense fluorescence. Perhaps the best way to introduce the semiconductor nanoparticles (NPs) or quantum dots (QDots) is by their visual appearance. These are core–shell NPs where the core is CdSe and the shell is ZnS. Another photograph of NPs with different sizes can be found. A wide range of emission wavelengths is available by changing the size or chemical composition of the NPs. The range of emission wavelengths has been extended to 4 μm using PbSe particles. PbSe QDots with emission wavelengths near 2 μm display quantum yields as high as 25%.

Spectral Properties of QDots

QDots display several favorable spectral features. The emission spectra of homogeneously sized QDots are about twofold more narrow than typical fluorophores. This feature can be seen by comparing the emission spectra of cyanine dyes with QDots (Figure 4). Additionally, the QDots do not display the long-wavelength tail common to all fluorophores. These tails interfere with the use of multiple fluorophores for imaging or multi-analyte measurements. The emission spectra of the QDots are roughly symmetrical on the wavelength scale and do not display such tails. Many of the commonly used organic fluorophores display strong long-wavelength absorption, but much less absorption at shorter wavelengths. For example, Cy3 and Cy5 are essentially non-absorbing at 400 nm (Figure 4). In contrast, the QDots absorb at these shorter wavelengths. This spectral property allows excitation of a range of NP sizes using

a single light source, which is needed for practical multiplex assays. The wide absorption spectra also allow excitation with a spectrally wide light source. The QDots also have large extinction coefficients (Ɛ) that on a molar basis can be up to tenfold larger than rhodamine. Small QDots have Ɛ values similar to that of R6G, near 200,000 M^{-1} cm^{-1}. Larger QDots can have Ɛ values as large as 2 x 10^6 M^{-1} cm^{-1}. And, finally, QDots can be highly photostable, making them useful probes for fluorescence microscopy.

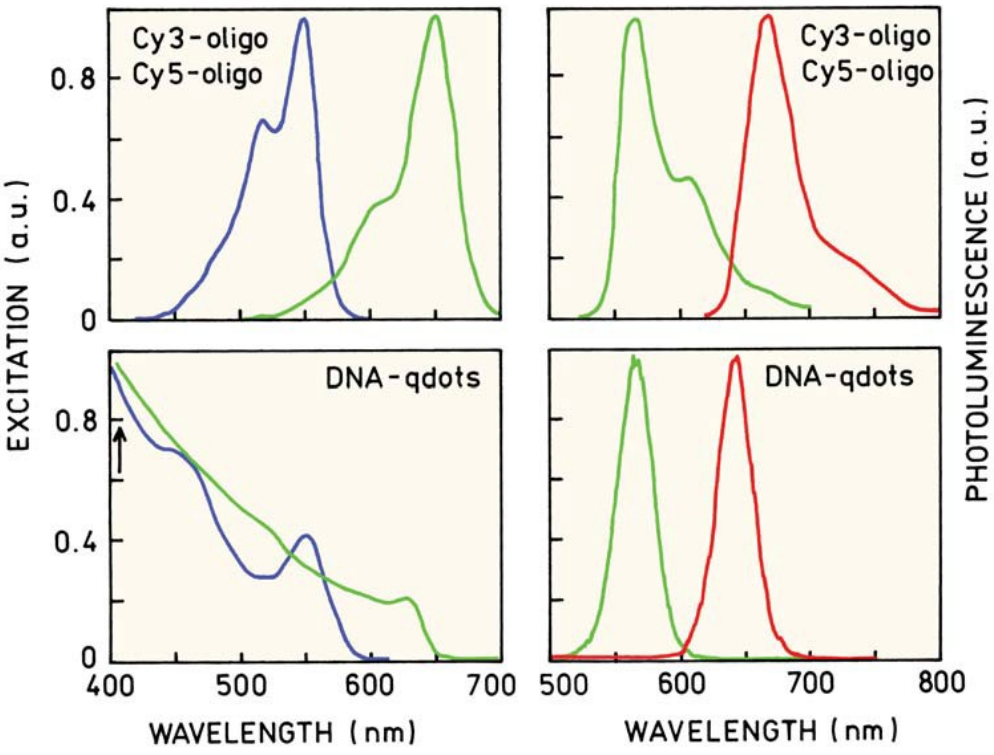


Figure 4. Absorption and emission spectra of Cy3- and Cy5-labeled oligomers and QDots with bound oligomers.