

Fluorescence Quenching & the Stern-Volmer Plot

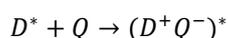
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Key Points

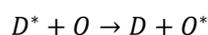
- Fluorescence quenching is a powerful mechanism that can be used to sense different interactions between molecules in a system.
- The Stern-Volmer plot is used to analyse how the fluorescence intensity or the fluorescence lifetime of an emitter is affected by the quencher's concentration.
- The plot is an important tool to characterise the different types of luminescence quenching (dynamic or static) in a bimolecular system.
- The quenching efficiency of a quencher towards a specific emitter can be determined using a Stern-Volmer plot.
- There are three main mechanisms associated with fluorescence quenching: FRET, Dexter, and PET.

What is Fluorescence Quenching?

A fluorescence quencher is a chemical species that increases the deactivation rate of an emitter molecule from its excited state. The quenching process is non-radiative and leads to a reduction in light emission from the population of excited molecules. This may involve either a transfer of charge or a transfer of energy from the excited emitter molecule – often named the *donor* (D^*) – to the quencher molecule (Q) (which is also commonly named the *acceptor* (A)). Many molecules can act as quenchers such as nitroaromatic compounds, gold nanoparticles and carbon nanotubes.



or



Quenching Types

Considering quenching as a bimolecular process between a quencher and an emitter, three different processes could be responsible for an observed decrease in emission intensity:

- Dynamic quenching
- Static quenching
- Trivial quenching

Dynamic Quenching

Dynamic quenching, also called collisional quenching, depends upon the diffusion of an emitter or a quencher through the medium. A decrease in emission intensity is due to the enhancement of the non-radiative deactivation rate from collisions between the emitter and quencher molecules. Since collisional quenching affects the excited state of an emitter, the photoluminescence lifetime (τ_P) changes with the introduction of different concentrations of quencher into the system while the absorption spectrum is not affected.

Static Quenching

Static quenching is a non-diffusion-mediated process that happens when the emitter and quencher interact, forming a new non-emissive species. The emission intensity of the system is quenched due to a reduction in the number of available emitter molecules. The absorption spectrum likely changes, but the fluorescence lifetime of the excited state is not affected.

Trivial Quenching

Trivial quenching is associated with measurement artefacts, such as the inner filter effect due to concentration changes and scattering.

The Stern-Volmer Plot

The Stern-Volmer plot can give quantitative information both on static quenching and on the kinetics of dynamic fluorescence quenching. The plot shows the ratio of the emission intensity as a function of the quencher's concentration $[Q]$, described by Eq. 1:

$$\frac{I_0}{I} = 1 + K_{SV} [Q] \quad (1)$$

where I_0 and I are, respectively, the intensity in the absence and presence of a quencher and K_{SV} is the Stern-Volmer constant. The constant gives information about the sensitivity of the system and, for dynamic quenching, is given by Eq. 2:

$$K_{SV} = k_q \tau_0 \quad (2)$$

where τ_0 is the photoluminescence lifetime of the emitter in the absence of a quencher, and k_q is the bimolecular quenching constant, which is an important parameter that shows how efficiently the quencher suppresses the emission intensity of the system, and its value is typically in the range of $10^9 - 10^{10} \text{ M}^{-1}\text{s}^{-1}$.^{1,2} For systems showing only dynamic quenching, K_{SV} can be determined from the slope of the Stern-Volmer plot, as shown in Figure 1.

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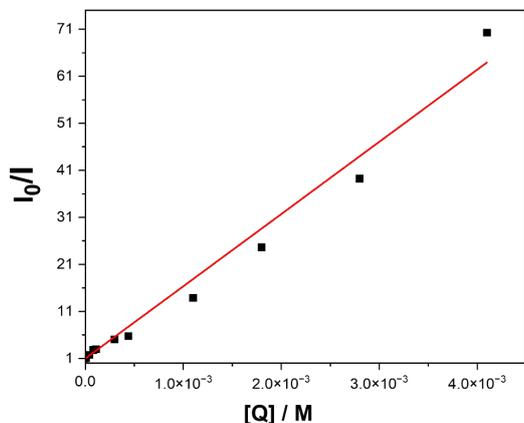


Figure 1. Example of a Stern-Volmer plot of the system Tris(2-phenylpyridinato) iridium(III) (fac-Ir(ppy)₃) and a nitroaromatic quencher (2,4-dinitrotoluene) showing dynamic quenching. The slope provides the Stern-Volmer constant.

Since the relative change in intensity $\frac{I_0}{I}$ is proportional to a corresponding change in fluorescence lifetime, Eq. 1 can also be represented as a function of lifetime (Eq. 3). This allows a dynamic quenching interaction to also be characterised from changes in the photoluminescence lifetime as a function of the concentration of the quencher. Therefore, the Stern-Volmer constant, K_{SV} , and bimolecular quenching constant, k_q , can be determined as a function of changes in lifetime with quencher concentration (Figure 2).

$$\frac{\tau_0}{\tau} = 1 + K_{SV} [Q] = 1 + k_q \tau_0 [Q] \quad (3)$$

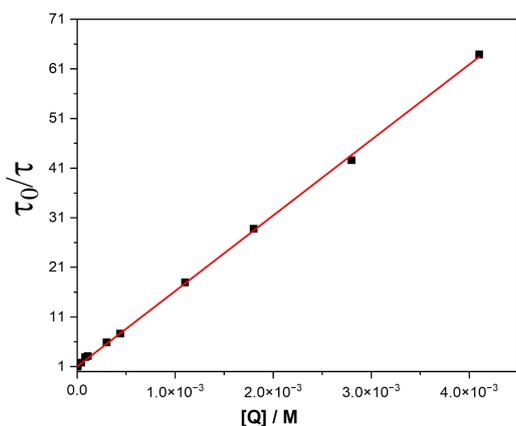


Figure 2. Example of a lifetime Stern-Volmer plot of the system composed by fac-Ir(ppy)₃ and 2,4-dinitrotoluene.

Non-linear deviation in the Stern-Volmer Plot

A linear relationship in the Stern-Volmer (SV) plot is the most common result obtained from fluorescence quenching. However, upward, or downward deviation behaviour is also possible (Figure 3).

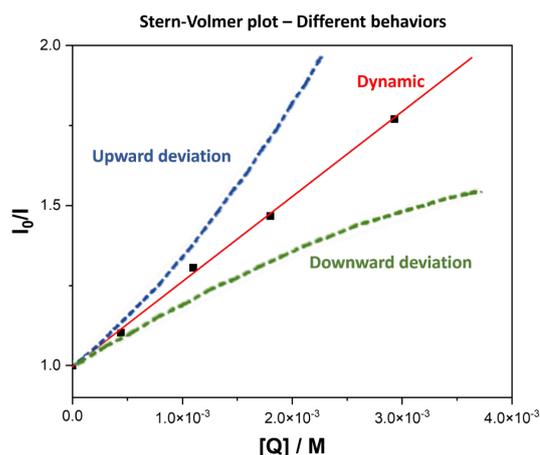


Figure 3. Example of a Stern-Volmer plot for quenching systems showing linear relationship (red curve); upward deviation relationship (blue curve); and downward deviation (green curve).

The linear relationship is often associated with dynamic quenching, where the quenching is mainly due to collisions between the emitter and quencher molecules. However, the linear relationship on the SV plot can also be associated with a purely static quenching system. Since static quenching does not show changes in the emission lifetime as a function of the quencher concentration, to distinguish between dynamic and static quenching processes, lifetime measurements should also be conducted.¹ The upward/positive deviation is a behaviour shown by systems in which both static and dynamic quenching are responsible for the decrease in the emission intensity. A downward/negative deviation is generally associated with the presence of two emitters with different SV constants, e.g., for the same concentration of quencher, the emitters will be quenched at different rates. In most cases, at lower concentrations of quencher, a linear relationship is evident; however, as the quencher concentration increases, the relationship becomes non-linear.

Quenching Mechanisms

Förster Resonance Energy Transfer (FRET)

Förster resonance energy transfer is a quenching mechanism where two molecules with similar excited-state energies exchange energy via a dipole-dipole resonance interaction. When a donor molecule, i.e. the emitter, is photoexcited (D^*), an oscillating dipole is generated. If the acceptor molecule, i.e. the quencher, (A) is in close proximity to the donor (typically between 1 and 10 nm), the dipole of the donor induces a dipole in the acceptor molecule, leading to the deactivation of the donor and the excitation of the acceptor.

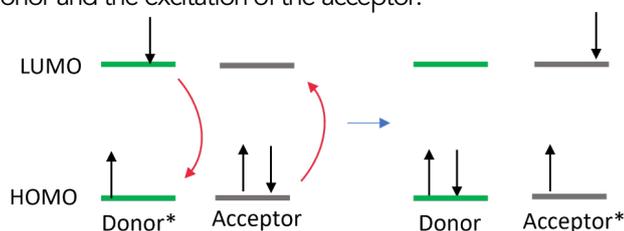


Figure 2. Molecular orbital diagram for Förster resonance energy transfer. Excited state molecules are represented by *.

There are two main requirements for FRET to occur (Figure 5). The first is a spectral overlap between the donor emission and the acceptor absorption, which is quantified as the spectral overlap integral, J . Second, the donor and acceptor molecules must be in close proximity because the interaction strength (for point dipoles) drops off as $1/r^6$, and the efficiency of energy transfer (η), can be expressed by Eq. 4:

$$\eta = \frac{R_0^6}{R_0^6 + r^6} \quad (4)$$

Where r is the centre-to-centre distance between the donor and the acceptor, R_0 is the Förster radius (distance at which the transfer efficiency is 50%) and is defined as Eq.5.

$$R_0 = [8.785 \times 10^{-5} (\kappa^2 n^{-4} \Phi^D J)]^{1/6} (\text{\AA}) \quad (5)$$

Where κ^2 is the orientation factor; n is the refractive index of the medium containing the donor and acceptor and Φ^D is the photoluminescence quantum yield of the donor in the absence of acceptor.

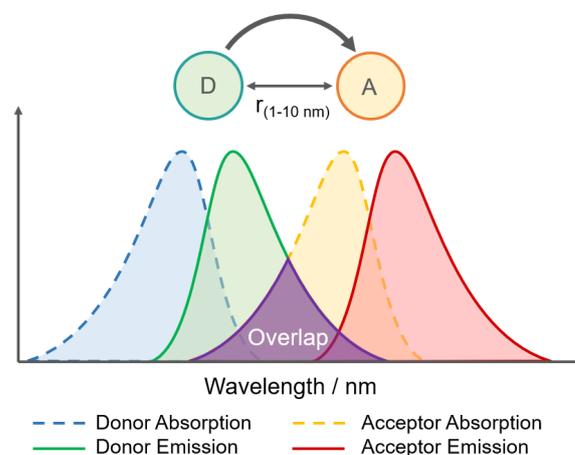


Figure 3. Criteria for FRET to occur.

Dexter Energy Transfer

Dexter energy transfer, also called electron exchange energy transfer, is a mechanism where a simultaneous exchange of electrons occurs between donor and acceptor molecules. In Dexter energy transfer, an electron is transferred from the HSOMO (Highest Singly Occupied Molecular Orbital) of the donor in its excited state to the LUMO (Lowest Unoccupied Molecular Orbital) of the acceptor in its ground state while an electron is transferred from the HOMO (Highest Occupied Singly Occupied Molecular Orbital) of the acceptor to the LSOMO (Lowest Singly Occupied Molecular Orbital) of the donor. At the end of the process, the acceptor molecule becomes excited, and the donor is deactivated. The mechanism is schematically explained in Figure 6. It is important to highlight that unlike FRET, in Dexter energy transfer it is possible to have a triplet excited donor producing a triplet excited state of the acceptor.

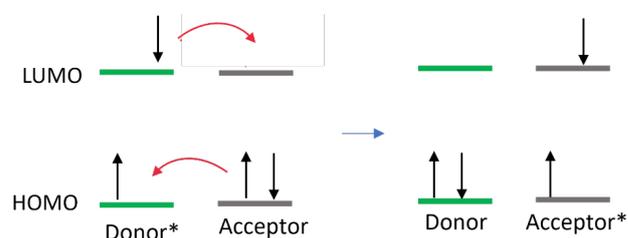


Figure 6. Molecular orbital diagram for Dexter energy transfer. Excited state molecules are represented by *.

Dexter energy transfer is also dependent of the spectral overlap between the donor emission and the acceptor absorption. However, as it requires orbital overlap, this means that Dexter energy transfer has a stronger dependence on the distance between donor and acceptor molecules than FRET. The probability falls off exponentially as the distance increases, according to Eq. 6:

$$k_{DET} = K J \exp\left[\frac{-2r}{L}\right] \quad (6)$$

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where k_{DET} is the rate constant of Dexter energy transfer, K is an experimental factor related to the orbital interactions, r is the distance between donor and acceptor and L is the sum of Van der Waals radius. The distance dependence on the efficiency of FRET and Dexter energy transfer is illustrated by Figure 7.

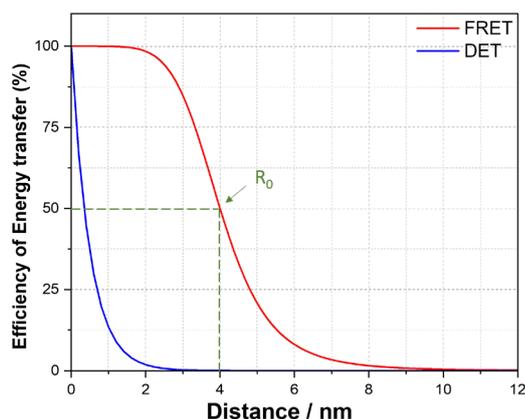
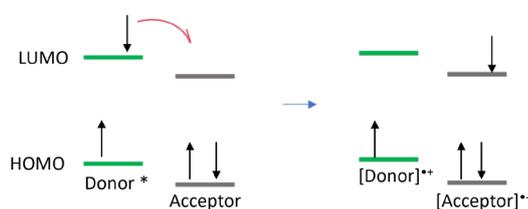


Figure 7. FRET and Dexter energy transfer mechanisms dependence on distance.

Photoinduced Electron Transfer (PET)

In the photoinduced electron transfer mechanism (Figure 8) an electron is, most commonly, transferred from the excited state donor to the ground state acceptor after photo-excitation. The process leads to the formation of a radical cation of the donor and a radical anion of the acceptor (Figure 8a). However, it is also possible to have a photoinduced "hole transfer" from the photoexcited donor to the acceptor, i.e. an electron transfers from the ground state acceptor to the excited state donor, leading to the formation of a radical anion of the so-called donor ($D^{\bullet-}$) and radical cation of the so-called acceptor.

a)



b)

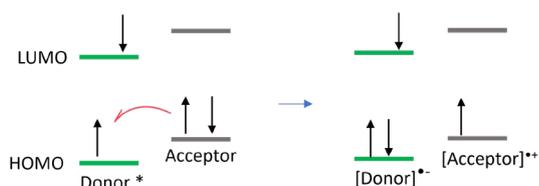


Figure 8. Molecular orbital diagram for photoinduced electron transfer. Excited state molecules are represented by *. (a) The electron transfer process from donor to acceptor. (b) Electron transfer process from the so-called acceptor to the ground state of the so-called donor (often described as a photoinduced hole transfer from donor to acceptor). Here the term donor is used to refer to the molecule initially in the excited state (the emitter).

PET can occur through space or through bonds; however, this requires orbital overlap between donor and acceptor, therefore the distance between donor and acceptor is an important factor that influences the electron transfer rate constant.

When an emitter is photoexcited in solution, the capability to accept or donate electrons changes, which leads to a decrease in the total energy of the charge transfer complex $[D^+A]^*$, in comparison with the initial system. This change in energy is expressed by the Rehm-Weller equation:

$$\Delta G = E(D^+/D) - E(A/A^-) - E_{00} - \frac{e^2}{\epsilon d} \quad (7)$$

where ΔG is the free energy change for PET. The $E(D^+/D)$ is the redox potential of the donor and $E(A/A^-)$ is the redox potential of the acceptor. E_{00} is the energy associated with the photoexcitation of the emitter from S_0 to S_1 . The last component of the equation expresses the Coulombic attraction energy experienced by the pair of ions following the electron transfer reaction where e is the elementary charge ($1.6 \times 10^{-19}C$); ϵ is the dielectric constant of the solvent; and d is the distance between charges.

About the Author



Michele Duarte Tonet was born in Brazil. She graduated in Physics and did her Master's in Materials Science and Engineering in her home country. Currently, Michele is a Physics PhD student at the Organic Semiconductor Centre at University of St Andrews (Scotland). She is co-supervised by Prof. Graham Turnbull (School of Physics and Astronomy) and Prof. Eli Zysman-Colman (School of Chemistry). Her PhD project is part funded by Edinburgh Instruments and is focused on the study of different classes of emitters for the development of optical sensors with high selectivity.

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2. Demchenko, A.P., Introduction to Fluorescence Sensing. 2015, Ukraine: Springer Dordrecht.



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