

Paths to Förster's resonance energy transfer (FRET) theory

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Abstract. Theodor Förster (1910–1974) developed a phenomenological theory of nonradiative resonance energy transfer which proved to be transformative in the fields of chemistry, biochemistry, and biology. This paper explores the experimental and the theoretical antecedents of Förster's theory of resonance energy transfer (FRET). Early studies of sensitized fluorescence, fluorescence depolarization, and photosynthesis demonstrated the phenomena of long-range energy transfer. At the same time physicists developed theoretical models which contained common physical mechanisms and parameters: oscillating dipoles as models for the atoms or molecules, dipole-dipole coupling for the interaction, and a distance R_0 that is optimal for resonance energy transfer. Early theories predicted R_0 that was too large as compared to experiments. Finally, in 1946 Förster developed a classical theory and in 1948 he developed a quantum mechanical theory; both theories predicted an inverse sixth power dependence of the rate of energy transfer and a R_0 that agreed with experiments. This paper attempts to determine why Förster succeeded when the other theoreticians failed to develop the correct theory. The putative roles of interdisciplinary education and collaborative research are discussed. Furthermore, I explore the role of science journals and their specific audiences in the popularization of FRET to a broad interdisciplinary community.

1 Introduction

Theodor Förster, who was a professor of physical chemistry at the Max-Planck Institute for Physical Chemistry in Göttingen (1947–1951), developed a phenomenological theory of nonradiative resonance energy transfer (FRET) which proved to be transformative in the fields of chemistry, biochemistry, and biology. Förster built upon the previous theories of his predecessors and first developed his FRET theory in classical form [Förster 1946, 1951] and then in quantum mechanical form [Förster 1948].

This paper analyzes the antecedents to his theory and the paths that led to *Förster's theory of resonance energy transfer* (FRET). I discuss the physicists'

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experiments on energy transfer in the gas phase, the anomalous concentration dependence of fluorescence depolarization in solution, and the biologists' experiments on photosynthesis; all these experiments converged on the existence of long-range energy transfer and led to the development of theories that describe energy transfer mechanisms.

The progression of theories showed a strong continuity in many of their assumptions and approximations; they eventually led to FRET. The role of specific journals in the dissemination of FRET to disparate scientific communities is analyzed in terms of their language and audience. The synergy of interdisciplinary education and research and the role of collaborative research are highlighted in the development of FRET and alternative theories of energy transfer. Finally, FRET is put into context among disparate theories of energy transfer [Förster 1965, 1967].

Perusal of the publications of Förster's predecessors demonstrates their use of dipole-dipole interactions and resonance concepts which he cited in his publications (Kallmann and London 1929; Perrin and Choucroun 1929; Perrin 1932, 1933). Kallmann and London derived an R^{-6} dependence for the rate of energy transfer as a function of the donor D and the acceptor A separation distance R ; however, the Perrins derived an R^{-3} dependence. But, all of their theories predicted a distance R_0 , where the probability of de-excitation by energy transfer is equal to the probability of de-excitation by all other mechanisms, that exceeded the experimentally derived R_0 .

What distinguished Förster's theories of FRET from the previous theories of resonance energy transfer that were developed by others [Förster 1946, 1948]? Förster's theories of FRET predicted the R^{-6} distance dependence for the rate of energy transfer, and predicted the value of R_0 that agreed with experiment. Perhaps his unique development of FRET was that he connected the rate constant for FRET with parameters that could be obtained from spectroscopic measurements of the donor D and the acceptor A.

Förster's genius was in his ability to transform a theory of resonance energy transfer that is based on the *Born-Oppenheimer approximation*, *very weak electronic coupling*, *allowed electronic transitions*, spectral line shapes, and *Franck-Condon factors or integrals*, into his phenomenological theory (FRET). Förster's readily measured *spectral overlap* between the donor and the acceptor molecules incorporated the above mentioned terms of molecular spectroscopy. This development facilitated the migration of FRET from the domains of physicists, physical chemists, and spectroscopists to the domain of biochemists and biologists.

Gregory Scholes elucidated the historical origin of the term *resonance energy transfer* (RET) which was also called "transfer by inductive resonance" [Scholes 2003]. The word "inductive" refers to the fact that the electronic interaction that promotes the energy transfer is due to a coupling of the transition moments of the donor D and the acceptor A. This coupling occurs via a Coulombic interaction and is analogous to the energy transfer between two coupled oscillators [Scholes 2003].

The following general publications are useful as an introduction to *resonance energy transfer* [Andrews and Demidov 1999; Scholes and Ghiggino 1994; Van der Meer et al. 1994; Wu and Brand 1994]. Robert Clegg wrote several comprehensive reviews on FRET [Clegg 1995, 1996, 2006]. Förster's theory is confounded by being presented in disparate equations by various authors and even in Förster's own papers [Braslavsky et al. 2008].

The following references are notable for their high pedagogical value to those who wish to obtain a clear and a deeper understanding of both Förster's original theories of resonance energy transfer and the approximations on which it was based, and the limitations of theories of FRET. Gregory D. Scholes has written an excellent review on FRET and electronic coupling for singlet-singlet, triplet-triplet, and superexchange mediated energy transfer [Scholes 2003]. David Beljonne and coworkers published a

critical article on the limits and the failure of FRET for various examples of energy transfer in biological and nanoscale systems [Beljonne et al. 2009]. These authors present a generalized Förster theory of RET and they consider both nonequilibrium processes and coherences in their theory. While the dipole approximation is an integral part of the history of resonance energy theories of energy transfer, the ideal dipole approximation fails when the D and the A are “too close” to each other [Muñoz-Losa et al. 2009].

FRET has wide applications in chemistry. It is a useful technique to investigate the shapes of polypeptides and protein molecules [Grinvald, Haas, Steinberg 1972; Hass, Katchalsky-Katzir, Steinberg 1978; Haas and Steinberg 1984; Haugland, Yguerabide, Stryer 1969]. FRET is also widely used in the development of FRET-based sensors. FRET is also very useful in the study of cellular processes which have been investigated with genetically encoded FRET biosensors: e.g. a recent publication describes a FRET based lactate sensor in single cancer cells [San Martín, Ceballo, Ruminot, Lerchundi, Frommer, and Barros 2013].

There are many varieties of FRET and it is a popular technique with many biological applications; however, the limitations of FRET techniques are often ignored or understated. In order to optimally use FRET techniques it is critical to understand their range of applicability and inherent limitations. A notable paper contains a comprehensive systemic catalog of 22 FRET imaging techniques with applications to multidimensional microscopy techniques which reviews applications that range from single molecule studies to live cell studies is a recommended source [Jares-Erijman and Jovin 2003].

While there are a variety of intensity based techniques to measure FRET, it is important to understand the advantages and the limitations of each technique. A critical comparison of these various techniques that highlights their strengths and weaknesses and their biological applications was recently published [Zeug, Woehler, Neher and Ponimaskin 2012]. FRET measurement can also be confounded by the presence of free donors and acceptors. A method, lux-FRET, that is based on linear unmixing of the spectral components offers a solution to this problem [Włodarczyk, Woehler, Kobe, Ponimaskin, Zeug, Neher 2008].

In general, it is difficult to distinguish electron transfer from FRET if the acceptor does not fluoresce, which is the case with heme proteins. Tryptophan is often used to study protein structure and dynamics, such as protein folding, as a donor in FRET studies. A recent publication raises the question of whether electron transfer pathways that can compete with FRET occur in a larger class of proteins [Consani, Auböck, van Mourik, and Chergui 2013; Winkler 2013]. The authors investigated the excited state decay pathways of the tryptophan amino acid residues in ferric myoglobins. They found that the more distant tryptophan (Trp7) relaxes by energy transfer to the heme, but the Trp14 excitation predominantly decays by electron transfer to the heme. The latter electron transfer has a quantum yield of more than 60% and outcompetes the FRET process [Consani, Auböck, van Mourik, and Chergui 2013].

1.1 Definition of terms

Nonradiative energy transfer refers to energy that is transferred between atoms or molecules or within a molecule; this transfer, from the energy *donor* D to the energy *acceptor* A, occurs without the radiation being first emitted from one atom or molecule and subsequently absorbed by a second atom or molecule. This process requires an interaction between the donor and the acceptor molecules; it is a resonance process and requires that some of the vibronic levels of the donor and the acceptor

are approximately equal in their energies. Förster's mechanism involves dipole-dipole interactions [Förster 1946].

Radiative energy transfer is due to a single process or a series of successive processes in which a photon is emitted from one atom or molecule and then absorbed by another atom or molecule. Radiative energy transfer does not depend on any interaction between the molecules involved in the transfer; it depends on their spectral overlap. Measurements of the emission properties of the donor molecule can be used to differentiate between nonradiative energy transfer and radiative transfer [Förster 1959].

Energy migration or excitation transport refers to a repetitive process in which the excitation is propagated over several molecules. The *efficiency of an energy transfer process* is the ratio of the rate of energy transfer divided by the rate of intramolecular deactivation of the donor in the excited state. Several physical processes can result in deactivation of the donor in the excited state, but for an individual molecule in the excited state a single deactivation process can only occur.

The term *FRET* is understood to be the acronym of "Förster's resonance energy transfer". This is consistent with the suggestion of Clegg, and the *IUPAC glossary of terms* [Braslavsky 2007; Braslavsky et al. 2008; Clegg 2006]. The earlier use the term FRET of the acronym "Fluorescence Resonance Energy Transfer" which occurred in papers related to the life sciences is incorrect; the energy transfer is nonradiative (not transferred by fluorescence) and can also involve triplet-singlet intermolecular energy transfer [Bennett et al. 1964; Braslavsky et al. 2008]. FRET refers to nonradiative energy transfer in which the resonance energy transfer is mediated by coupled Coulombic dipole-dipole interaction.

Two terms are commonly used in the equations that describe FRET. The *separation distance* between the donor D and the acceptor A is denoted by the symbol R . The term R_0 is the *Förster radius* (in his 1946 publication he called it the critical molecular separation), which is the distance between the donor D and the acceptor A molecules at which rate constant k_T for energy transfer and the rate constant for deactivation of the donor D excited state by all other mechanisms are equal; R_0 is typically in the range of 1–10 nm and is solvent dependent [Braslavsky et al. 2008; Förster 1969b; Knox and Amerongen 2002]. At the *Förster radius* the FRET efficiency is 50%. Förster called the term a critical transfer distance and wrote "[when] $R = R_0$ there are equal probabilities for [energy] transfer and for internal deactivation of the primarily excited [donor] molecule by a radiative or a nonradiative process" [Förster 1960b].

As defined in the IUPAC Compendium of Chemical Technology, "an electromagnetic wave may induce an oscillating electric moment in a molecule (possibly leading to absorption if the oscillation frequency is equal to the light frequency). The amplitude of this moment is the transition moment between the initial (i) and final (f) states (here assumed to be non-degenerate):

$$\vec{\mu}_{if} = \langle f | \vec{\mu}_{op} | i \rangle$$

where $\vec{\mu}_{op}$ is the electric dipole moment operator, a vector operator that is the sum of the position vectors of all charged particles weighted with their charge. The transition moment $\vec{\mu}_{if}$ is a vector in the molecular framework, characterized both by its direction and its probability [McNaught and Wilkinson 1997]. The transition moment mixes different states and is a measure of a transient charge displacement during the transition. For symmetric planar molecules, e.g. naphthalene, pyrene, perylene, and anthracene the absorption transition moment is either parallel or perpendicular to the long axis of the molecule and differs for the type of transition, $S_0 \rightarrow S_1$ or $S_0 \rightarrow S_2$ and for the particular molecule.

The equations for FRET include the term κ^2 , the square of the *orientation factor*, which describes the relative orientation of the transition dipole moments of the D and

the A. Förster defined the *orientation factor* κ ,

$$\kappa = \cos \phi_{DA} - 3 \cos \phi_D \cos \phi_A$$

where ϕ_{DA} is the angle between the transition moment vectors of both molecules, D and A, ϕ_D and ϕ_A are the angles between these respective vectors and the vector from one molecule to another is \vec{r} . For free rotation of D and A, the assumed average value of κ^2 is two-thirds, and measurements of total depolarization of the emission are used to validate this assumption. But this assumption can be invalid [Braslavsky et al. 2008].

While FRET measurements are very useful to detect changes in the conformation of large molecules its use as a “spectroscopic ruler” to measure distances is limited by our knowledge of the relative orientation of the donor D, and the acceptor A transition dipole moments [Dale, Eisinger and Blumberg 1975]. The square of the orientation factor κ^2 can vary between the limits of 0 and 4. These authors have developed a technique that is based on depolarization measurements and gives the upper and the lower limits for the average of the square of the orientation factor $\langle \kappa^2 \rangle$ [Dale, Eisinger and Blumberg 1975].

A notable signature of the FRET mechanism of energy transfer between D and A molecules, is that the lifetime of the donor D is decreased¹. In the intermolecular energy transfer process there are two cases: (1) D and A are identical, and (2) D and A are different molecules. For the first case the energy can transfer repetitively in both directions. For the second case of different molecules the energy transfers in one direction from D to A; the energy of the excited state of the donor D is greater than the energy of the excited state of the acceptor A. The donor D is excited by light and the excitation energy is transferred to the acceptor A; *simultaneously* the donor D returns to the ground state. This process does not involve collisions and the transfer can occur over several molecular diameters with high efficiency. Intramolecular energy transfer can also occur by FRET, i.e. energy transfer in a protein molecule.

The Förster mechanism of vibrational-relaxation resonance energy transfer invokes both the *Oppenheimer approximation* and the *Franck-Condon Factors or Integrals*. Another name for the *Born-Oppenheimer approximation* is the *adiabatic approximation* [Born and Oppenheimer 1927; Eckart 1935]. This follows from the fact that in electronic transitions the masses of the nuclei are much greater than the masses of the electrons, and therefore electronic transitions occur on a much shorter time scale than rearrangements of the nuclei. This explains why we draw diagrams of electronic transitions as vertical transitions; the nuclei remain in fixed positions during the duration of the electronic transitions. The electronic transition occurs between two wave functions that have the greatest overlap integral of all the vibronic states in the upper electronic state. A *vibronic transition* is an electronic transition that derives its intensity from the vibration of a molecule.

In the *Born-Oppenheimer approximation* the total wave function for a molecule is set as a product of two factors at a fixed position of the nuclei. The wavefunction for a molecule is expressed as the product of two terms: the electronic components and the nuclear (vibrational) components:

$$\Psi = \Psi_{\text{electronic}} \times \Psi_{\text{nuclear}}.$$

¹ Fluorescence lifetimes could be measured with the lifetime fluorometer [Gaviola 1927].

The first factor is a function of the positions of the electrons $\vec{r} = (\vec{r}_1, \dots, \vec{r}_m)$ and the second the positions of the nuclei $\vec{R} = (\vec{R}_1, \dots, \vec{R}_n)$.

$$\Psi(\vec{r}_1, \dots, \vec{r}_m, \vec{R}_1, \dots, \vec{R}_n) = \Psi_{\text{electronic}}(\vec{r}_1, \dots, \vec{r}_m, \vec{R}_1^{\text{fixed}}, \dots, \vec{R}_n^{\text{fixed}}) \\ \times \Psi_{\text{nuclear}}(\vec{R}_1, \dots, \vec{R}_n).$$

Förster assumed the *Born-Oppenheimer approximation* in his derivation of his quantum mechanical theory when he separated the electron and the nuclear coordinates in the wave functions.

The *Franck-Condon principle* is a related concept [Condon 1926; Franck 1926]. When a molecule undergoes a transition from the ground state to a higher electronic state this transition results in a higher vibronic state that closely resembles the vibronic state of the lowest electronic state. We consider an electronic transition between a state at electronic level E and vibronic level ν to a state at electronic level E' and vibronic level ν' . The primed quantities represent the excited states. The symbol $*$ denotes the complex conjugate. The transition moment can be expressed as:

$$\vec{\mu} = -e \int \int \Psi_{el'}^*(\vec{r}) \Psi_{vib'}^*(\vec{R}) \vec{r} \Psi_{el}(\vec{r}) \Psi_{vib}(\vec{R}) d^3\vec{r} d^3\vec{R} \\ \vec{\mu} = -e \int \Psi_{el'}^*(\vec{r}) \vec{r} \Psi_{el}(\vec{r}) d^3\vec{r} \cdot \int \Psi_{vib'}^*(\vec{R}) \Psi_{vib}(\vec{R}) d^3\vec{R}.$$

The second factor of the last equation is:

$$S_{\nu, \nu'} = \int \Psi_{vib'}^*(\vec{R}) \Psi_{vib}(\vec{R}) d^3\vec{R}.$$

The quantity $S_{\nu, \nu'}$ is the *overlap integral* between the initial vibrational wavefunction and the final vibrational wavefunction. The magnitude of this integral is a measure of the similarity between the vibrational wavefunctions of the initial and the final states. The intensity of a transition from the vibrational level ν in the ground electronic state to the vibrational level ν' of the excited state is proportional to the square of the transition dipole moment. Therefore, the intensity of this transition is proportional to $S_{\nu, \nu'}^2$. The *Franck-Condon factor or integral* for the transition between $E, \nu \rightarrow E', \nu'$ is $S_{\nu, \nu'}^2$. The larger the *Franck-Condon factor*, the larger the absorption intensity of the transition.

FRET incorporated the *Franck-Condon factor or integral* into his phenomenological *spectral overlap integral* denoted by the symbol J . The degree of spectral overlap between the emission of the donor D and the absorption of the acceptor A is expressed as the *spectral overlap integral*. This quantity is measured for each D and A pair.

$$J = \int_0^\infty F_D(\lambda) \varepsilon_A(\lambda) \lambda^4 d\lambda \quad \text{in terms of wavelengths and} \\ J = \int_0^\infty F_D(\tilde{\nu}) \varepsilon_A(\tilde{\nu}) \frac{d\tilde{\nu}}{\tilde{\nu}^4} \quad \text{in terms of wave numbers.}$$

The emission spectrum of the donor D, with respect to wavelength and wavenumber, is normalized to one.

$$\int_0^{\infty} F_D(\lambda) d\lambda = 1 \quad \text{with respect to wavelength, and}$$

$$\int_0^{\infty} F_D(\tilde{\nu}) d\tilde{\nu} = 1 \quad \text{with respect to wavenumber.}$$

The integral of the absorption spectra is not dependent on the choice of scale for both the wavenumber scale and the wavelength scale because the extinction coefficient is independent of the scale:

$$\varepsilon(\tilde{\nu}) = \varepsilon(\lambda).$$

But the same is not true for the emission spectra, which differs depending on the selection of either the wavenumber or the wavelength scale:

$$F_D(\tilde{\nu}) \neq F_D(\lambda)$$

and

$$F_D(\tilde{\nu}) = \lambda^2 F_D(\lambda).$$

1.2 Ancillary themes related to the development of FRET and its dissemination

Within this historical account are several secondary themes which may be pertinent to readers with an interest in the history and sociology of science. First, I contextually analyze FRET in terms of alternative theories. Then, I describe a succession of antecedent theories of RET that were incorrect in both their mathematical formulation and in some of their underlying physical assumptions. I review the concept of resonance. Second, I analyze in detail Förster's development of both his classical and then his quantum mechanical theory of FRET. Third, I discuss the classifications of resonance energy transfer in terms of coupling strength. Fourth, I describe the different validation experiments for FRET and their consequences. Finally, I give examples that demonstrate the important role of research in photosynthesis in the formation of FRET.

I pose the following questions: why did Förster's theory yield the correct experimental results in contrast to the previous theoretical works of others? And why did he correctly understand the physical phenomenon in contrast to his predecessors? How did his interdisciplinary education and training lead to his successful theory? Furthermore, I present an example that illustrates three points: the power of interdisciplinary research and education, the power of collaborative research from disparate fields. These points are well illustrated by the productive collaboration of a biophysicist and a theoretical physicist [Arnold and Oppenheimer 1950].

Photosynthetic research was central to Förster's research interests. The experimental studies on photosynthesis that were published in the years prior to the development of Förster's FRET theories were known to Förster as he cited them in his 1946 classical papers, in subsequent papers, and in his famous text book. He acknowledged the influence of these prior studies on his interest in nonradiative energy transfer [Emerson and Arnold 1932a,b; Emerson and Lewis 1942; Förster 1946, 1951; Gaffron and Wohl 1936a,b]. Two disparate groups of scientists, the biologists and the physicists, each accumulated experimental data that suggested the putative existence of still unknown mechanisms of long-range nonradiative energy transfer. Förster was

able to bridge these fields and with his interdisciplinary knowledge develop his theory of FRET that was applicable to a variety of disciplines.

Today Förster's FRET techniques are one of the most popular and important tools in biology and biochemistry as evidenced by the number of publications and citations. FRET is used to measure intermolecular and intramolecular interactions and it can be used on living cells. FRET provides researchers with an optical technique to measure changes in molecular conformation with nanometer resolution. But these features alone cannot explain FRET's great popularity and utility in modern scientific research; its popularity also required its dissemination across disparate scientific disciplines; i.e. physics, physical chemistry, biochemistry, and biology.

One can ask the following critical question: how did FRET migrate from the domain of physicists, spectroscopists, and physical chemists to the domain of biochemists and biologists? What is the effect of publishing in a particular journal with a specific readership in the communication of new theories to readers from disparate disciplines? The first experimental validation of the efficiency of FRET as a function of intramolecular distance within a molecule was performed in 1965. However, it was published in the *J. Am. Chem. Soc.* [Latt, Cheung, and Blout 1965]. This journal was read predominately by chemists. Although the publication was clean and elegant it did not reach the wide audience of biologists. This situation dramatically changed with the subsequent paper authored by a different research group and had a title with the key words: *A Spectroscopic Ruler*. It was published in *Proc. Natl. Acad. Sci. USA* which was accessible to biologists and the combination of the title and the particular journal made Förster's theory of RET popular with biologists [Stryer and Haugland 1967]. This paper validated the technique, and it proposed the use of FRET to study both inter- and intramolecular interactions on a scale of molecular dimensions.

Another factor in the wide dissemination of FRET is the choice of language. Förster's early publications were in German, but later Förster published English reviews of his energy migration theory [Förster 1959, 1960a,b, 1965]. These papers are important because they made FRET theory accessible to the English speaking scientific community.

Furthermore, we can ask what other lessons for the history of science can be learned from the history of resonance energy transfer? If we look at the scientists who contributed to the development of resonance energy transfer they can be classified as physicists; albeit some of them such as the Perrins did work with molecules in solution and today may be considered to be physical chemists, but the others were grounded in physics with minimal training in chemistry. The educational system in the 1930s and the 1940s traditionally separated these two branches of science. Thus, it is not surprising that these physicists, with the exception of Francis Perrin, made theoretical models for RET that assumed exact resonance and ignored the fact that molecular spectra in solutions consist of broad bands, and that the excited state of the donor D molecule undergoes thermal relaxation from a higher vibronic state to the lower vibronic state of the first electronic state; a process that involves interaction between the donor D molecules and the molecules of the solvent.

Förster was the first to correctly interpret these effects and to correctly incorporate them in his FRET theory [Förster 1946, 1948, 1951]. Perusal of Förster's publications and his seminal book, *Fluoreszenz Organischer Verbindungen*, clearly demonstrate his deep knowledge of chemistry, and in particular molecular spectroscopy. Another critical factor was his deep interest in energy transfer that occurs in biological systems, in particular the role of energy transfer in photosynthesis. I posit that his interdisciplinary education that encompassed both physics and chemistry, together with his long term interest in understanding the role of energy transfer in photosynthesis, were the two factors which resulted in his unique success in formulating both his classical and his quantum theory of FRET.

1.3 FRET in context among disparate theories of energy transfer

This article discusses development of Förster's resonance energy transfer theory and its antecedents, but it is important to state that there are alternative theories of energy transfer that are appropriate for specific experimental conditions. Many inorganic systems demonstrate energy transfer mediated by forbidden transitions; i.e. triplet-triplet transitions, and in these cases the Förster's theory is totally inapplicable. For these cases where forbidden transitions predominate Dexter developed a theory of sensitized luminescence in solids that accounts for exchange effects [Dexter 1953].

How does FRET compare with Dexter's theory? Förster's theory of energy transfer is specific to singlet-singlet energy transfer and Dexter's theory of energy describes triplet-triplet energy transfer. Förster's theory is based on dipole-dipole Coulombic interaction and the exchange interaction is neglected; Dexter's theory is based on exchange interaction [Dexter 1953; Scholes 2003]. Förster's theory has a rate of energy transfer that is proportional to the inverse sixth power of the separation distance R , of the donor D and the acceptor A. Dexter's theory of exchange induced energy transfer has a rate of transfer that decreases as:

$$\exp\left(\frac{-2R}{L}\right),$$

where L is the sum of van der Waals radii of the donor D and the acceptor A. For Förster's dipole-dipole induced energy transfer the rate depends on the oscillator strengths of the transitions; however, for Dexter's exchange-induced energy transfer the rate is independent of the oscillator strengths of the donor D and the acceptor A.

A new generalized Förster-Dexter theory of intramolecular energy transfer for systems which have discrete or quasicontinuous initial and final states has been developed [Lin, Xiao, and Dietz 1993]. Instead of expressing the rate constant for energy transfer in terms spectral overlap these authors used molecular parameters such as electronic energy gap and free energy change. They investigated the effects of the energy-gap dependence and the effects of temperature on the rate of energy transfer.

Perusal of the literature on FRET demonstrates a wide range of applications, instrument developments, and software developments. A recent comprehensive review article that contains 216 references on the physics of energy transfer and new and fundamental developments in the theory is highly recommended [Olaya-Castro and Scholes 2011]. They provide an elegant proof to rule out the trivial mechanism of energy transfer. "It was established that the primary process involved in transferring excitation energy is not a trivial mechanism whereby donor fluorescence is reabsorbed by an acceptor. That is easily proved because energy transfer can happen with a quantum efficiency of unity, which is impossible if the wavevector of the transferred photon can take any direction, like it does in fluorescence" [Olaya-Castro and Scholes 2011].

These authors discuss several effects that go beyond the standard Förster theory and they point out new directions of research. They carefully evaluated the recent theoretical and experimental studies that incorporate both the coherent and the incoherent quantum dynamics that are critical to energy transfer in molecular aggregates. They discuss recent research to find experimental signatures of coherent superpositions of electronic states that are involved in energy transfer. In particular they describe energy transfer mechanisms in a variety of multichromophoric systems: conjugated polymers, supramolecular assemblies, fluorescence-based sensors, DNA, and in some light-harvesting antenna complexes such as the components of the photosynthetic apparatus. The authors ask the following critical questions: "(1) What mechanisms are responsible for persistent electronic coherence in the systems studied so far? (2) How can the microscopic details of the biomolecular environment be probed? (3) Do coherent dynamics help energy transfer under sunlight illumination? (4) How

might we put quantum-coherent energy transfer to use to improve other light-driven phenomena in chemistry and physics?" [Olaya-Castro and Scholes 2011].

2 Early studies of energy transfer at the University of Göttingen

2.1 Institutes of Physics at the University of Göttingen

In the nineteen twenties the Institutes of Physics at the University of Göttingen were good examples of interdisciplinary research which also characterized Förster's research. In that period the University of Göttingen was a center of theoretical and experimental physics as well as mathematics. How did this focus of excellence develop? The physicist Woldemar Voigt initiated the strong tradition of theoretical physics in Göttingen. Voigt was made a full professor for theoretical physics as well as the director of the mathematical physics institute in 1883, and he was made the co-director of the physical department of the mathematical-physical seminar [Jungnickel and McCormmach 1986].

In 1920, when Debye left Göttingen his replacement was the theoretician Max Born. Born requested the ministry of culture to split Debye's former department into two departments, one for theory and another for experiment [Jungnickel and McCormmach 1986]. Born helped his friend James Franck, an experimentalist, to become an ordinary professor in the adjacent institute. In 1921 three institutes were created: Robert Pohl directed the First Institute of Physics, James Franck directed the Second Institute of Physics, and Max Born directed the Institute for Theoretical Physics. For the case of Born and Franck we see a synergistic interaction between theory and experiment that proved so productive for their research [Lemmerich 2007; Masters 2013].

2.2 Energy transfer in gases

Collision experiments were very important in the early understanding of energy transfer processes and they preceded the investigations of energy migration or transfer in solutions and in condensed matter. In 1913 James Franck and Gustav Ludwig Hertz performed energy transfer experiments in which they showed energy transfer occurred between colliding high-speed electrons and atoms [Franck and Hertz 1914]. These authors defined this process as: *collisions of the first kind*; there was a conversion of translational energy into electronic energy. These collisions produced excited state atoms and electrons that had lower velocities.

In 1921 Klein and Rosseland posited the reverse process between excited atoms and electrons that resulted in the atoms returning to the ground state and the electrons acquiring kinetic energy [Klein and Rosseland 1921]. In the process of the excited atom returning to the ground state there is no emitted radiation, and the energy of the transition, $h\nu$, is distributed between both colliding particles as kinetic energy. They denoted radiationless conversion of electronic energy into kinetic energy as the result of collisions is defined as: *collisions of the second kind* [Klein and Rosseland 1921].

2.3 Sensitized fluorescence

Franck expanded the definition of *collisions of the second kind* to include collisions between atoms [Franck 1922]. In this case an atom, A^* , in the excited state collides

with an atom, B, in the ground state. The result is atom, A, in the ground state, and atom, B*, in the excited state. The superscript * indicates an excited state. Franck postulated that a gas of mercury atoms in the excited state can collide with atoms of a different element in the ground state with the result that the mercury atoms are excited and then they emit their excitation energy as fluorescence. Franck maintained his interest in energy transfer between molecules and mentored his student Günther Cario. Cario and then Franck and Cario confirmed these predictions for several metallic elements in the vapor phase [Cario 1922; Cario and Franck 1922, 1923].

It is instructive to recall these experiments on the indirect excitation of thallium fluorescence in a mixture of mercury vapor [Cario and Franck 1922]. They introduced mercury vapor and thallium vapor into a quartz tube at a specific pressure and temperature. The mean-free path between collisions and the kinetic energy of the atoms was determined at a fixed temperature. These parameters could be varied by changing the temperature of the quartz tube. Therefore, it was possible to alter the kinetic energy of the atoms by varying the temperature of the quartz tube, which was contained in an electric furnace. The quartz tube was illuminated with the emission line at 2536.7 Å from a water-cooled, quartz-mercury lamp. A spectroscope was used to analyze the light emitted from the quartz tube. The emission from the tube was determined to be from the thallium atoms.

Cario and Franck posited the following mechanism: the mercury atom absorbed the 2536.7 Å radiation, which induced a transition from the lower energy ground state to the upper energy level excited state. Upon collision with a thallium atom the excited mercury atom transferred its energy to the thallium atoms, exciting the thallium atoms to the excited state, which then emitted their characteristic radiation, and in the process they returned to the ground electronic state. In the absence of the mercury atoms in the quartz tube, the thallium atoms were not excited by the 2536.7 Å radiation.

The theoretical requirement for this energy transfer to occur between the two different atoms is that the time between collisions is the same order of magnitude as the mean lifetime of the excited state of the mercury atom. The excitation of the mercury atom is due to the absorption of the radiation. The fact that the thallium atom acquired a large velocity from collision with the excited state of the mercury atom is easily verified by measuring the Doppler shift on the emission line of the thallium atom. The high velocity following the collision would result in a line broadening due to the Doppler shift. This type of mechanism was named *sensitized fluorescence* (*Sensibilisierte Fluoreszenz*), in analogy to the sensitization of photographic plates, since the mercury atoms sensitized the thallium atoms to the 2536.7 Å light from the mercury lamp. Similar results were obtained if silver atoms replaced the thallium atoms in the quartz tube. They also obtained similar results in experiments with other alkali metals and mercury atoms in the gaseous state.

Cario and Franck expanded their studies in 1923 when they demonstrated that the energy transfer occurs simultaneously and not in a sequential mechanism involving two separate steps [Cario and Franck 1923]. Their conclusion is based on the measurement of the intensities of the thallium emission lines and the measurement of Doppler broadening of emission line widths. The significance of these experiments is that they indicated the existence of energy transfer between two atoms in the gas phase that occurred over large distances; distances that exceeded their collision radii. Franck and Jordan summarized all the experimental findings on collision processes and electronic excitation up to 1926 in their book *Anregung von Quantensprüngen durch Stösse* (*Excitation from Quantum Jumps through Collisions*) [Franck and Jordan 1926].

3 Early publications on dipole-dipole interactions

3.1 Dipole-dipole interactions and their relation to FRET

The concept of an *electric dipole* is extremely useful and we see its utility in the early theories of resonance energy transfer and later in FRET. The following introductory material is based on textbooks of modern electromagnetic theory [Brau 2004; Jackson 1999; Purcell and Morin 2013].

The goal is to calculate the energy of interaction between two dipoles. There are two steps to the calculation: the first step is to calculate the potential at point r due to the electric dipole at the origin, and the second step is to calculate the electric field that is derived from that potential. Then the energy of interaction, U , of the two dipoles is calculated as the scalar product of the electric field and the electric dipole $\vec{\mu}_2$.

The dipole consists of two electric charges, $+q$ and $-q$, separated by a distance d

$$\vec{\mu} = qd.$$

There are two electric dipoles, $\vec{\mu}_1$ and $\vec{\mu}_2$. The first electric dipole is on the origin of the coordinate system and the second electric dipole is located at a distance r from the origin.

The potential at point r which is due to the electric dipole located at the origin is:

$$\Phi(\vec{r}, \theta) = \frac{1}{4\pi\epsilon_0} \frac{\vec{\mu}_1 \cdot \vec{r}}{r^2}$$

where ϵ_0 is the permittivity of free space, θ is the angle between the axis of the dipole and the radius vector to the point (x, y, z) . The electric field that results from this potential is:

$$\vec{E}(\vec{r}) = -\vec{\nabla}\Phi(\vec{r}) = \frac{1}{4\pi\epsilon_0} \left(3 \frac{\vec{\mu}_1 \cdot \vec{r}}{r^5} \vec{r} - \frac{\vec{\mu}_1}{r^3} \right).$$

The energy of interaction, U , between these two dipoles is:

$$U = -\vec{E}(\vec{r}) \cdot \vec{\mu}_2 = \frac{1}{4\pi\epsilon_0} \left[\frac{\vec{\mu}_1 \cdot \vec{\mu}_2}{r^3} - 3 \frac{(\vec{\mu}_1 \cdot \vec{r})(\vec{\mu}_2 \cdot \vec{r})}{r^5} \right].$$

Several important points should be noted. First, the electric field that is derived from a pair of equal and opposite charges with a separation distance of r has the identical electric field distribution as an electric dipole at point $R(x, y, z)$ if the separation of the two charges is much less than the distance R from the charges. Alternatively, if $R \gg r$, then the electric field distribution from the two charges can be replaced with the electric field distribution of an electric dipole. This result is valid for any distribution of electrical charges for which the sum of all the charges is zero. Second, the potential of an electric dipole decreases as \vec{r}^{-2} . The electric field strength \vec{E} of the dipole decreases as \vec{r}^{-3} . Third, the spatial distributions of the electric field from both a static electric dipole and that from an oscillating electric dipole are identical. Forth, the energy of interaction between two electric dipoles is a function of two factors: the *distance of separation* between the electric dipoles, and the *relative orientation* of the two electric dipoles $\vec{\mu}_1$ and $\vec{\mu}_2$.

The field of an oscillating dipole depends on the distance from the dipole [Jackson 1999]. The dipole is oscillating at the frequency ω . The observer's distance from the oscillating dipole is r , and λ is given by:

$$\lambda = \frac{2\pi c}{\omega}.$$

There are special zones where the electromagnetic field has different properties [Jackson 1999]. In the near zone, $d \ll r \ll \lambda$, the electromagnetic field does not propagate or radiate. It has the properties of a static field with both transverse and longitudinal components.

FRET occurs when the acceptor A is located in the *near zone* of the donor D. The static electric field varies harmonically and the acceptor A gains energy from the field of the donor D. This process increases the rate of decay of the excited state of the donor D; the lifetime of the donor D is decreased in this nonradiative energy transfer mechanism.

3.2 Early publications on dipole-dipole interactions

Dipole-dipole interactions were posited as the basis of molecular interactions in 1912 when Willem Hendrik Keesom (1876–1956) developed the first mathematical description of dipole-dipole interactions. Keesom suggested that the interaction of two permanent molecular dipoles could induce one molecule to orientate with respect to the other one [Keesom 1912]. Some details of Keesom's theory of dipole-dipole interactions are given below. Keesom derived the formulas for various types of dipole-dipole interactions. He showed that the average interaction energy, $\langle U \rangle$ of two freely rotating dipoles, $\vec{\mu}_1$ and $\vec{\mu}_2$ that are separated by the distance, R , is given by

$$\langle U \rangle = -\frac{2}{3} \frac{\vec{\mu}_1^2 \vec{\mu}_2^2}{(4\pi\epsilon_0)^2 kTR^6}$$

where ϵ_0 is the vacuum permittivity, k is the Boltzmann constant, and T is the absolute temperature. At the same time Peter Debye developed classical models of molecular interactions that were based on the mutual interaction between two molecules; one molecule has a permanent dipole and the other molecule had an induced molecular dipole [Debye 1920, 1921].

3.3 Early publications on resonance energy transfer

For a long time resonance energy transfer mechanisms were also referred to as: *transfer by inductive resonance* [Scholes 2003]. In the years prior to the 1929 seminal publication of Kallmann and London's quantum mechanical theory of RET there appeared several publications that described classical theories which pointed to the occurrence of energy transfer over distances that were larger than the collision radii of the atoms [Holtmark 1925; Mensing 1926; Nordheim 1926]. These studies indicated that physical collisions were not necessary for energy transfer to occur between atoms. Therefore, they concluded there must be non-collisional mechanisms of long-distance energy transfer between atoms or molecules.

Analysis of collision cross-sections and their quenching efficiency yielded a parameter, the spectroscopic cross-section, which produced another part of the puzzle of RET. From the kinetic theory of gases the radii of the two colliding atoms can be calculated. In many cases the spectroscopic cross-section was larger the sum of the two collision radii calculated from kinetic theory. This provided evidence that energy transfer could occur over distances that were larger than the sum of the collision radii, and therefore collisions between atoms are not a necessary condition for the occurrence of energy transfer.

Holtmark, who worked in Trondhjem (Trondheim), published a classical mechanics paper that reported on investigations of the width of the absorption lines of sodium

atoms in the gas phase [Holtmark 1925]. It was observed that the spectral widths of these absorptions were broader than expected from collisions modeled on the collision theory of gases. It was assumed that the atoms could be modeled as oscillating dipoles, and that the interaction of the two atoms was proportional to the product of their oscillator strengths. Furthermore the atom-atom interaction was modeled as dipole-dipole interactions and therefore Holtmark calculated that the interaction between two dipoles would be proportional to R^{-3} where R is the interatomic separation. Holtmark did not make the assumption of exact resonance between the two atoms and he estimated the combined interaction by an overlap integral.

Another pertinent publication by Lucy Mensing, who worked in Göttingen, described her theory of spectral line broadening [Mensing 1925]. Mensing assumed the interaction between two atoms could be modeled by dipole-dipole interactions, and building on the Bohr-Sommerfeld theory of elliptical electron orbits she derived a line broadening that was proportional to R^{-3} [Eckert 2013].

Lothar Nordheim working in Göttingen published a classical theory of how collisions induce excitation in atoms [Nordheim 1926]. His theory modeled collisions between atoms in which the form of the interaction was dipole-dipole interactions. He modeled energy transfer between colliding atoms that were proportional to powers of their transition moments, and he calculated this for different multipole interactions. Prior to Nordheim's publication Heisenberg published his first paper on quantum theory, but Nordheim chose the classical modeling for simplicity. Kallmann and London cited all of these author's publications in their seminal publication of a quantum mechanical model for energy transfer [Kallmann and London 1929].

These three papers contributed common features to the understanding of long-range resonance energy transfer between molecules and to the development of theoretical mechanisms. First, the spectroscopic cross-sections were larger than the collisional cross-sections that could be calculated from classical kinetic theory of gases. That hinted of long-range molecular energy transfer. Second, the use of dipole-dipole interactions could facilitate energy transfer between molecules that are separated by long distances. Third, it was also shown that another factor was significant in the energy transfer process: the requirement of overlap between the spectroscopic oscillator strengths of D and A.

3.4 Kallmann and London's quantum mechanical theory of energy transfer

Sometimes the collaboration of two or more scientists results in a seminal contribution. This was the case for the joint work of Hartmut Kallmann² and Fritz London³ who

² Hartmut Kallmann (1896–1978) studied physics at the University of Göttingen and in 1920 received his doctorate under the supervision of Max Planck. He then worked at the Kaiser Wilhelm Institute for Physical Chemistry and Electrochemistry in Berlin where he collaborated with Fritz Haber and Fritz London. In 1933 Kallmann was removed from his position because he was a non-Aryan. He remained in Berlin through the Second World War where he worked for IG Farben. In 1948 he immigrated to the United States where he worked for the United States Army Signal Corps at Fort Monmouth, New Jersey for one year and then he became a professor of physics in the physics department of New York University [Reisman 2006].

³ Fritz London (1900–1954) is known for his work with Walter Heitler at the University of Berlin in which they used the newly developed quantum mechanics to explain the chemical bonding in molecular hydrogen [Gavroglu 1995]. Their theory evolved into the valence bond approach in quantum chemistry. In 1930 London worked with Rudolph Eisenschitz and formulated a model of the interaction of two atoms of a Noble gas that attract each other at large distances and repel each other at short distances; the interaction is eponymously

developed their quantum mechanical theory of energy transfer while they worked in Berlin.

The first quantum mechanical theory of energy transfer between atoms that are separated at distances larger than their collisional radii set the stage for future developments of RET [Kallmann and London 1929].

3.5 Details of the Kallmann and London's 1929 publication

In 1929, Kallmann and London published a quantum mechanical theory of resonance energy transfer between atoms in the gas phase [Kallmann and London 1929]. Their theory consisted of a dipole-dipole interaction term and the term R_0 . At the time of their collaboration London was working at the Institute for Theoretical Physics at the University of Berlin. This publication is significant since it was the first quantum mechanical theory of energy transfer and was incorporated into the theory of Francis Perrin for the case of solutions, and was subsequently extended by Förster. Their paper was written only three years after Schrödinger published his four classic papers on wave mechanics in 1926 [Gavroglu and Simões 2011; Jammer 1989; Kragh 2002; Mehra and Rechenberg 2001].

In 1927, Schrödinger published a paper in which he detailed the quantum mechanical treatment of energy transfer between two atoms [Schrödinger 1927]. Schrödinger showed that for two identical atoms with very narrow energy states, the mathematical solution derived by using the method of variation of constants is that of an oscillating system in which the energy successively is transferred in both direction between the two atoms. Kallmann and London's quantum mechanical treatment of energy transfer between two coupled oscillators is based on the 1927 publication of Schrödinger and also to the works of Dirac [Dirac 1926, 1927; Schrödinger 1927].

Kallmann and London organized their paper into two parts, a general part and a specialized part, and included an appendix on the spectral line broadening due to collisions. The general part of their paper included a discussion of quantum mechanical resonance, the quantum mechanical theory of energy exchange between two atoms, the elementary processes of energy excitation, the kinetic theory of energy excitation, and the limiting case of exact resonance. In the general introduction to the prior work Kallmann and London carefully credited the independent publications of Mensing, Holtzmark, Nordheim. The specialized parts include discussions of excitation through collisions for specific mixtures of atoms, depolarization of resonance radiations, and quantum mechanical excitation and chemical reaction kinetics.

For energy transfer, Kallmann and London assumed the condition of approximate resonance between the energy levels of the interacting atoms in the gas phase (i.e., no vibronic relaxation) and used second-order perturbation theory to calculate the energy of interaction. The authors assumed each atom has two states and the differences in energy between these two states were not equal, but approximately the same for the two atoms.

Kallmann and London gave the quantum mechanical interaction term in terms of classical Coulomb interaction $U_{\alpha\beta}$ for the dipole interaction of two oscillators α and β [Dirac 1926, 1927; Schrödinger 1927].

The quantum mechanical expression is:

$$U_{\alpha\beta} \propto \frac{(\vec{\mu}_\alpha \cdot \vec{\mu}_\beta)}{R^3}$$

called the London dispersion force [Heitler and London 1927; London 1928]. Fritz London made important contributions to two fields of research: the theory of the chemical bond and to the theory of intermolecular interactions which were important in understanding both superconductivity and superfluidity [Eisenschitz and London 1930; London 1930, 1937].

where $\vec{\mu}$ is the respective transition dipole moments for the respective atoms α and β , and R is previously defined (see Sect. 1.1). This expression is of the same form as the classical expression for the interaction energy of two dipoles.

The use of second-order time-dependent perturbation theory with this interaction energy gives the R^{-6} dependence and the transition matrix expressions. Kallmann and London derived the anomalously large spectroscopic cross-section for their model of the energy transfer process by integrating the above equation over the variable R .

Kallmann and London's theory defined the constant R_0 and the authors derived the R^{-6} dependence for energy transfer between two atoms that have slightly unequal energy levels between their ground and excited states. Their calculation of the spectroscopic cross section for the dipole-dipole mediated energy transfer between two atoms, one in the excited state and one in the ground states gave values that were larger than those derived from collision cross sections. In their calculations the authors showed that the dipole-dipole interactions between the two atoms have anomalous cross-sections: their calculated spectroscopic cross-section for atomic collisions, based on their model, was much larger than the collision cross-section that kinetic theory predicted. That result presented further evidence for the existence of long-range resonance energy transfer between atoms. The English subtitle of their publication is: *a contribution to the problem of anomalously large cross-sections*. Note that the authors *did not assume exact resonance*, but their theory predicted values of R_0 that were too large compared to experimental data.

4 Concepts of resonance

Resonance is fundamental to FRET. In this section I describe the use of the word *resonance* in physics and chemistry before Förster coined the term: *resonance energy transfer*. The resonance concept was central to *Franck's Principle* in which Franck posited the excited states of two atoms exhibit an *energy resonance* which facilitates the resonance energy transfer between two colliding atoms [Franck 1922]. Thus, in 1922 the connection between resonance as defined by Franck and the mechanism of long-range energy transfer was known.

Further empirical evidence in support of the idea that the efficiency of energy transfer between colliding atoms depends on a resonance phenomenon began to accumulate in the late 1920s. In a critical study Beutler and Josephy demonstrated that *collisions of the second kind* occur with a high probability only if the energy of the electronic transition from the ground state to the excited state of atoms "A" and "B" are approximately equal [Beutler and Josephy 1927, 1929]. In order to achieve a high probability of energy transfer between two atoms or between two molecules, it is necessary that the electronic transition energies between the ground state and the excited state must be approximately equal. The concept of resonance energy transfer incorporates this energy requirement.

The Swiss mathematician and physicist Leonhard Euler was the first to describe mechanical resonance of masses coupled by springs. Resonance phenomena in mechanical systems such as coupled pendulums are well known and taught in elementary physics courses [Fano and Fano 1959; Feynman et al. 2006].

Resonance also occurs with electromagnetic waves and in electrical circuits. Heinrich Hertz first demonstrated the properties of electromagnetic waves by constructing a source and a detector such that the source and the detector were in resonance [Masters 2011]. Resonance in LCR electrical circuits was known and exploited in radios for tuning to specific frequencies.

4.1 Heisenberg's theory of the many-body-problem and resonance

Recent works on the history of quantum mechanics discuss the various physicists and their experimental and theoretical works that led to modern quantum mechanics and its applications [Duncan and Janssen 2013; Jammer 1989; Kragh 2002; Mehra and Rechenberg 2001]. In 1925 Heisenberg developed his formulation of quantum mechanics-matrix mechanics, then Heisenberg, Pascual, and Born further developed the formalism of matrix mechanics and applied it to physical problems [Mehra and Rechenberg 2001].

Heisenberg in 1926 was the first physicist to describe the resonance-like phenomenon in quantum mechanics. After Heisenberg published his first papers on the matrix formulation of quantum mechanics he discovered the phenomenon of quantum mechanical resonance and addressed its applications in three papers on the many-body problem and resonance in quantum mechanics [Heisenberg 1926a,b, 1927].

Heisenberg applied both his matrix mechanics and Schrödinger's wave mechanics to solve the problem of two coupled oscillators; both methods yielded identical results. For the uncoupled oscillators each term is split by coupling of the oscillators. The eigenfunctions consist of a symmetric and an antisymmetric term. Heisenberg then applied the results of this paper to the helium atom and he concluded the necessity of using antisymmetric eigenfunctions to be consistent with the *Pauli Exclusion Principle*⁴ [Heisenberg 1926b]. In Heisenberg's third paper on resonance in quantum mechanics he applied the results of the two previous papers to the theory of multiplet spectra of atoms and molecules [Heisenberg 1927].

Heisenberg gave the first example from classical mechanics. Two coupled mechanical systems, for example two coupled pendulums are considered to be in resonance if their natural frequencies of oscillation are approximately the same. By analogy, Heisenberg then defined resonance in two quantum mechanical systems. He wrote that two atomic systems are in resonance when the frequency of absorption of one atom is approximately equal to the emission frequency of the other atom. For classical coupled mechanical systems that are in resonance energy can be transferred between the two oscillators. Similarly, for two atoms or molecules that are in resonance, there can be coupling between the two systems that will affect the energies and the spectra of the coupled system.

4.2 Fermi Resonance

In 1932 Fermi published a paper in which he described the excited vibrational states of polyatomic molecules as measured with infrared and Raman spectroscopy. He explained the shifts in the intensities of absorption spectra that were previously not understood [Fermi 1932]. *Fermi Resonance* can be described as a linear combination of the vibrations of each molecule. The two interacting states are treated by perturbation theory to give the energies of the combined interacting system.

Fermi Resonance usually occurs between the normal and the overtone modes of vibration; the vibrations must have the same symmetry, and the transitions between the ground state and the excited state must have approximately the same energies.

⁴ The *Pauli Exclusion Principle* states that no two identical fermions may occupy the same quantum state simultaneously.

4.3 Pauling and Wilson on resonance

In the 1920s a new field of quantum chemistry was developed. The early works on quantum mechanical resonance were interdisciplinary and bridged the fields of chemistry and physics [Gavroglu and Simões 2011].

Pauling and Wilson introduced the resonance phenomenon in their classic on quantum mechanics and its applications to chemistry [Pauling and Wilson 1935]. They present two examples from classical mechanics: a system of coupled pendulums and a system of two tuning forks attached to the same base. In both cases energy will be transferred from an oscillating object (one pendulum or one tuning fork) to the second object which will begin to oscillate as the amplitude of the first object decreases with time. Then the process reverses as energy is transferred from the second object back to the first object. The efficiency of the energy transfer is maximum when the natural vibration frequencies of both objects are approximately equal.

Pauling's resonance theory follows from Heisenberg's 1927 papers on resonance between two interacting harmonic oscillators and presents a concise description of resonance in quantum mechanics [Heisenberg 1927]. Pauling built upon Heisenberg's results: for the uncoupled oscillators each term is split by coupling of the oscillators and the eigenfunctions consist of a symmetric and an antisymmetric term.

It must be understood that while mechanical resonance refers to oscillations, the use of resonance in FRET and in quantum chemistry refers to linear combinations of states or chemical structures; the chemical structures that form the resonance structure do not oscillate among various structures. For example benzene can be viewed as two resonance structures, but the real molecule has equal bond lengths and does not oscillate between two forms of double chemical bonds and single chemical bonds.

5 The Perrins' theories on resonance energy transfer

Förster acknowledged his debt to both Perrins, the father Jean (1870–1942) and his son Francis (1901–1992), for being the first to note that energy could be transferred between one molecule and another molecule in the local environment and that this energy transfer was mediated by a direct electromagnetic interaction between the excited molecule and another nearby molecule [Förster 1948]. The Perrins, famous for their research in luminescence, developed several theories of molecular energy transfer and from their analysis of depolarization experiments they concluded that energy transfer occurs over distances that are much larger than molecular dimensions [Berberan-Santos 2001].

5.1 Jean Perrin's classical theory of 1927

Jean Perrin explained excitation transfer by resonance in terms of classical physics [J. Perrin 1927, 1936]. In his theory the atom is represented as a single oscillator with an electron (Hertz electric dipole) that oscillates at the frequency ν_0 . The oscillator can interact with another oscillator, identical to the first one, via the classical dipole-dipole interaction which was invoked in contemporary publications. These two oscillators are assumed to be in *exact resonance* at the same frequency of oscillation. If two identical electric dipoles are in proximity (in the *near field*) they can undergo resonance energy transfer. He labeled his resonance mechanism *transfert d'activation*.

In an analogy with two coupled mechanical pendulums in exact resonance, the two atoms would undergo successive reversible energy transfer from the first atom to

the second, and from the second atom to the first. The energy transfer would depend on the separation distance of the two atoms. Jean Perrin postulated that at large separation distances of the two atoms the emission would occur in the first atom. But for close proximity, he thought that the two atoms would have equal probability for emission of the excitation energy. At the critical distance R_0 the mutual coupling between the two atoms is equal to the coupling of an atom with the radiation field. For the assumption of the two atoms in exact resonance, Jean Perrin calculated that

$$R_0 \sim \frac{c}{\omega} \sim \frac{\lambda}{2\pi}$$

where the speed of light is c , and ω is the oscillator’s angular frequency, which is the eigenfrequency ν multiplied by 2π and the corresponding wavelength is λ which can also be considered as the wavelength of the emission of the donor D. Previously other researchers considered such resonance energy transfer processes [Holtmark 1925; Mensing 1926; Nordheim 1926].

In this model of energy transfer the rate of energy transfer, k_T , is proportional to the inverse third power of the separation distance of the two atoms. The rate of energy transfer is:

$$k_T \sim \frac{1}{R^3}.$$

Jean Perrin could quantitate the resonance energy transfer by measuring the amount of depolarization of the fluorescence emission. Jean Perrin’s theory of energy transfer was studied by the decrease of polarization of dye molecules in solution as a function of increasing concentration. As the concentration increased, the average distance between the dye molecules decreased and the enhanced probability of resonance excitation transfer decreased the measured polarization of the emitted luminescence.

Jean Perrin’s theory did not consider molecular vibrations nor the role of the molecules that comprise the solvent; he only considered the dielectric constant of the solvent. These effects broaden the sharp electronic transitions that occur at a single frequency into a broad frequency region. Jean Perrin’s theory also ignored the fact that there is an internal conversion between vibrational levels that occurs as a result of thermal relaxation. This process results in the *Stokes’ shift* between the absorption and the emission spectra. Upon excitation the molecule is in a higher vibrational level of the first electronic state; in the process of thermal relaxation the molecule relaxes to the lowest vibronic level of the same electronic state. This occurs by interaction with the vibrations of the solvent molecules.

5.2 Francis Perrin’s quantum mechanical theory of 1932, 1933

Francis Perrin’s⁵ quantum mechanical theory followed the same assumptions that Kallmann and London first used for their quantum mechanical model of resonance energy transfer in the gas phase [F. Perrin 1927, 1932, 1933]. Kallmann and London assumed very sharp electronic transitions; however this was later modified to include the line broadening effects of collisions and also Doppler effects

⁵ Francis Perrin, made several important contributions to the field of molecular luminescence: the active sphere model for quenching of fluorescence, the relation between the quantum yield and the lifetime of an excited state, the theory of fluorescence polarization, including the Perrin equation, which permitted the fluorescence lifetimes of dyes in solution to be measured, and the first qualitative theory of the depolarization of fluorescence by resonance energy transfer [F. Perrin 1925, 1926, 1927, 1929, 1931, 1932, 1933]. Francis Perrin’s key scientific writings have been published [Neveu and Baton 1998].

[Kallmann and London 1929]. This assumption led both Perrins to erroneously conclude that the rate of energy transfer is proportional to R^{-3} which predicted that energy transfer would occur at distances (200 Å) that exceeded those distances derived from experiments.

In 1932 Francis Perrin published a quantum mechanical theory of energy transfer between identical molecules in close proximity, first in a gas phase and then in modified form for molecules in solution [F. Perrin 1932, 1933]. In this work he extended the original theory of resonance energy transfer, based on classical physics, that was developed by Jean Perrin. The new theory of Francis Perrin also built on the previous theory of Kallmann and London; however, he extended their work by considering molecules in solution, taking account of vibronic interactions [Kallmann and London 1929].

It is instructive to further describe some of the details of the 1932 paper by Francis Perrin, which is based on exact resonance between two energy states: the ground state and the excited state. The theory applied quantum mechanics, which he termed *wave mechanics* after Louis de Broglie, to the following problem. One atom is initially in the excited state and the second atom is in the ground state at a distance from the first atom. The problem is to calculate the frequency or probability for the excitation energy of the first atom to be transferred to the second atom, as a function of the interaction energy between the two atoms, which depends on the distance of their separation. He extended the treatment for energy transfer between two fixed and separated atoms to the case of molecules in solution, which includes the perturbations of solvent molecules.

Francis Perrin included the condition of quantized transfer of energy between two atoms and gave the name *d'induction quantique* to this phenomenon. First he considers the case of two atoms, α and β , with the same atomic number that are separated by a great distance. A great distance is defined as the distance in which the energy of interaction between the two atoms is negligible (i.e., there is no interaction between the two atoms). He ignored vibronic relaxation processes. He wrote the time-independent Schrödinger equation for each atom. He then showed that for the combined system of atoms α the eigenfunctions and the eigenvalues can be expressed as linear combinations of the solution for each atom. The energies of excitation, *d'activation*, for each atom are equal, E_α equals E_β , and the total energy of the combined system is just the sum of the energies of each individual atom.

Second, Francis Perrin considered the case in which the two identical atoms are brought together to a distance R . He assumed that the separation distance is still large enough for the exchange of electrons between the two atoms, which resulted from the superposition of their electron clouds. Francis Perrin assumed that the two atoms, separated by the distance R , interact through a dipole-dipole interaction (similar to the theories of Kallmann and London, and that of Jean Perrin). The interaction term between the two atoms is based on electrostatic interactions of the oscillating charge densities on each atom. He then proposed that the interaction an excited atom and an atom in the ground state is based on a coupling in which the interaction is inversely dependent on the third power of the distance between the two atoms. Perrin simplified the perturbation theory for the degenerate case by invoking symmetry.

Third, he described the *transfer of excitation* or *transfert d'activation*. He suggested that this resonance energy transfer could occur if the two atoms or molecules were within the distance of $\frac{\lambda_0}{2\pi}$ where λ_0 is the wavelength of the oscillating electric field [F. Perrin 1932, 1933]. To describe this situation, he used the time-dependent Schrödinger equation for two identical atoms with the initial condition that one is in the excited state and one is in the ground state. The results of the calculation showed that the excitation will first be transferred from atom α to atom β , and then the excitation will be transferred from atom β to atom α . This oscillation of excitation

energy will continue to occur and is similar to the resonance between two coupled mechanical oscillators of the same mass, which represents a mechanical analog.

Fourth, Francis Perrin compared the lifetime for the resonance transfer of excitation energy between two identical atoms with the lifetime for spontaneous emission. He then defines a special separation distance R between atom α and atom β as R_0 . He defined the R_0 as that distance in which the time for resonance transfer is equal to the mean lifetime of the luminescence (fluorescence). Förster used this same definition in his first classical derivation of FRET [Förster 1946]. By definition, at the distance R_0 , the probability of de-excitation by energy transfer is equal to the probability of de-excitation by all other mechanisms, e.g. fluorescence, quenching. The term R_0 appears in the 1928 paper from Kallmann and London and in those of Jean Perrin.

Finally, Francis Perrin considered the case of resonance energy transfer between molecules in solution: in this case vibronic relaxation becomes possible. He considers the case of a very dilute solution of fluorescent molecules in which the very viscous solvent will make displacement of the molecules over short times negligible. For example, the effect of increasing concentration on the decrease of polarization [depolarization] in a solution of the molecule fluorescein in glycerin was investigated. By considering the effect of molecules in a solvent, Francis Perrin calculated the value of R_0 is $0.22\lambda_0$ [F. Perrin 1932, 1933]. This distance still exceeds the experimentally derived distance for energy transfer and therefore his theory is still not able to predict the experimental results.

Francis Perrin had a good insight to the physical problem and made the prescient statement that internal vibration of the two molecules and the vibrational interaction between the molecules and the solvent could alter the absorption and the emission bands. Furthermore, Francis Perrin noted (as did Kallmann and London), for molecules in solution at room temperature, that there is a broadening of the absorption and emission bands and the atomic line spectra are observed as broad bands.

6 Förster's resonance energy transfer [FRET]

6.1 Brief biography of Theodor Förster

Why did Förster succeed in developing his FRET theory for molecules in solution? Perhaps we can answer these questions by studying the educational and professional background of Förster. I followed the obituary of Förster written by Albert Weller who was a colleague of Förster, the biographical reflection on the work of Förster written by George Porter, and the more recent biography by Horst Kramer and Peter Fischer [Weller 1974, 1980; Porter 1976; Kramer and Fischer 2011].

Förster studied in Frankfurt and completed his *Abitur* in 1929. Afterwards he studied physics and mathematics at the University of Frankfurt am Main. Then he pursued his graduate work under the guidance of his mentor Erwin Madelung who was the Chair of Theoretical Physics from 1921 when Max Born resigned from that position and moved to Göttingen. Madelung was a theoretical physicist and worked in atomic physics and quantum mechanics. Before Förster completed his dissertation he worked as an *Assistant* to the physical chemist Karl Friedrich Bonhoeffer at the University of Frankfurt. In 1933 Förster received the degree of Doctor of Philosophy at the age of twenty three. The title of his thesis is: *Zur Polarisation von elektronen durch Reflexion*.

In 1933 Adolf Hitler became the Chancellor of Germany and in the same year Förster joined the Nationalsozialistische Deutsche Arbeiterpartei, (National Socialist German Workers Party) or the Nazi party (NSDAP) and the Stormtroopers

(*Sturmabteilung* or SA) which was the paramilitary organization of the Nazi Party [Klee 2003 p. 158]. These facts of Förster's life during the Third Reich are not mentioned in the obituary and the biographical articles that I previously cited [Weller 1974, 1980; Porter 1976; Kramer and Fischer 2011].

The social and political events that followed from 1933 greatly impacted the administration of the German universities and their continuing developments in theoretical physics [Lemmerich 2007; Reisman 2006]. A recent comprehensive biography of Arnold Sommerfeld, based on his correspondence as well as archival sources, provides a personal insight into these developments [Eckert 2013].

When Bonhoeffer moved to the University of Leipzig in 1934, where he became a full professor of physical chemistry and the director of the Institute of Physical Chemistry which was founded by Wilhelm Ostwald; Förster moved there with him. Förster completed his *Habilitation* in 1940 which gave him the right to teach at a university. At that time such luminaries as Werner Heisenberg, and Peter Debye also worked at the University of Leipzig. Kramer and Fischer credit Debye for teaching Förster how to apply theoretical physics to practical problems; a trait which is characteristic of Förster's publications [Kramer and Fischer 2011]. I posit that Bonhoeffer's research style, which was based on an interdisciplinary approach where physical chemistry techniques were used to investigate biological problems, had a great influence on Förster.

During Förster's tenure in Leipzig (1934–1942) he began a long series of investigations into the application of quantum mechanics to the spectroscopy of organic compounds. His great talent was to merge the abstract mathematical formulation of quantum mechanics with the visual structural formulas that organic chemists used to represent molecules. In a five year period Förster published a series of papers on light absorption of carbon compounds which resulted in a full professorship at age thirty two. It is reasonable to assume that Bonhoeffer⁶ and his colleagues facilitated the transition of Förster research focus from physics and mathematics to physical and photophysical chemistry that he elegantly exploited. According to Weller, one mission of Förster was to explain the new developments of quantum mechanics in a manner that could be understandable to chemists [Weller 1974, 1980]. In my opinion, the formulation of Förster's classical theory of FRET is a superb fulfillment of Förster's mission to make modern developments in physics useful and understandable to chemists.

In the years 1942 to 1945 Förster was a Full Professor at the State University of Posen (Poznań) and during these years he did not publish any scientific papers. However, during this period he collected material for a book on the absorption and fluorescence of organic compounds. While working at Posen he was able to apply physical principles to the interpretation of photochemical reactions of organic molecules [Kramer and Fischer 2011]. Again, he bridged the fields of physics and physical organic chemistry. Förster's interdisciplinary approach to molecular spectroscopy of organic compounds was both unusual at that time and prescient in that it showed the benefits of making intellectual bridges between dissimilar fields of study.

Three prescient publications indicate the themes that interested Förster in the years of 1946 and 1947. He published a paper on the topic of energy transfer and fluorescence: this paper had important applications both in the photochemistry of organic compounds and in the extremely complex and interesting process of photosynthesis – the process in which green plants use the energy of sunlight to synthesize carbohydrates [Förster 1946, 1947a,b].

⁶ In 1971 a new institute was formed, its current full name honors Bonhoeffer: *The Max Planck Institute for Biophysical Chemistry – Karl Friedrich Bonhoeffer Institute*.



Fig. 1. Theodor Förster.

In 1948 the Max-Planck Gesellschaft was formed from the restructured former Kaiser-Wilhelm Gesellschaft and two new institutes were founded in Göttingen; Werner Heisenberg directed the new physical institute and Bonhoeffer headed the new institute for physical chemistry. After the war, from 1947 to 1951, Förster again joined Bonhoeffer in Göttingen. From 1949 to 1951 Förster was a group leader in the department of *Chemical Structure Research* at the Max-Planck Institute for Physical Chemistry in Göttingen.

A major contribution of Förster during his work in Göttingen was the publication of his book *Floreszenz Organischer Verbindungen* [Förster 1951]. The title page of his seminal book *Floreszenz Organischer Verbindungen* gives his title and location as: Professor für physikalische Chemie, Max-Planck-Institut für physikalische Chemie, Göttingen [Förster 1951]. This book is an exemplar of how to integrate modern quantum mechanics and molecular spectroscopy of organic compounds in a manner that is both rigorous and understandable to the readers who presumably were chemists. This book written in German quickly became a classic. This is yet another example of Förster's interdisciplinary approach to science. While this seminal work was not translated in to English it played a major role in the education of a generation of physical chemists and physical organic chemists due to its clear exposition of the spectroscopy of organic compounds.

From 1951 Förster (Fig. 1) was a Full Professor at the *Technische Hochschule Stuttgart*. At Stuttgart his colleagues called Förster's *Floreszenz Organischer Verbindungen* the *Hausbibel* [house bible]. Because of Förster's international prestige and reputation his research group attracted an international following and this in turn attracted many photochemists from all over the world.

In a series of papers from 1960 to 1973 he continued to develop and refine his theory of FRET and in the process to make it more accessible to chemists, which was one of his primary missions [Weller 1974, 1980]. In addition to his seminal research into resonance energy transfer in organic compounds Förster also made significant contributions to photochemistry; for example his discovery of charge transfer complexes and his development of the Förster cycle which is a method to calculate changes in the equilibrium constant in the excited state. While at Stuttgart, Förster discovered that

a new phenomenon that explained the different fluorescence spectra of pyrene in dilute and in concentrated solutions. He proposed *excimers* as intermediates in photochemical dimerization reactions [Förster 1952, 1969; Weller 1974]. Excimers are dimers between an excited and a ground state molecule [Frenkel 1931a,b; Davydov 1962]. The molecules that form *excimers* do not form dimers if both molecules are in the ground state. Förster's corpus of work resulted in several nominations for the Nobel Prize in chemistry; an honor that he never received [Kramer and Fischer 2011].

6.2 Förster's classical resonance energy transfer theory

It was the seminal work of Förster in Göttingen that provided a phenomenological theory for resonance energy transfer. FRET is a brilliant extension of the earlier ideas of several physicists who worked on resonance energy transfer: J. Perrin, F. Perrin, H. Kallmann and F. London, and S.I. Vavilov.

Förster made his theory accessible to a wide range of scientists by grouping the complex physical terms of his theory into a few experimentally accessible parameters such as the overlap integral, the lifetime of the donor D, the refractive index of the medium through which the energy transfer occurs, and the orientation parameter for the D-A pair.

The Förster theory extracts from the experimental measurements the *transfer efficiency* and also the separation distance R between the donor D and the acceptor A, and the Förster radius R_0 . The transfer efficiency Φ_T is defined as the number of quanta transferred from the donor D to the acceptor A divided by the number of quanta that are absorbed by the donor D. The transfer efficiency can be expressed as:

$$\Phi_T = \frac{1}{1 + \left(\frac{R}{R_0}\right)^6}.$$

This equation is plotted in Figure 2. The plot indicates that the transfer efficiency is most sensitive to R when $R = R_0$. The transfer efficiency also is dependent on the relative orientation of the transition dipole moments of D and A.

Förster developed another possible method for the transfer of excitation between distant electronic systems: the mechanism of *resonance transfer* or transfer by *inductive resonance* [Förster 1960a]. These mechanisms are the basis of sensitized fluorescence in atomic or molecular systems.

Förster published two versions of the classical theory of resonance energy transfer [Förster 1946, 1951]. In his 1946 paper he did not include the orientation factor κ and the refractive index factor n in his equations. His second classical theory developed in his 1951 book *Fluoreszenz Organischer Verbindungen* included these two parameters.

My perusal of Förster's publications shows several characteristics that make it difficult to deconstruct and to decipher the assumptions and his reasoning for each step in his derivations. My study of the corpus of his work, both in German and in English, also shows that he repeated much of the content and the equations, but the explanations of his reasoning is extremely sparse and thus his concise derivations without his explanations for each bold step in the derivations results in a recondite understanding. In spite of these difficulties, where feasible, I attempted to fill in the lacunae with my plausible explanations. Further difficulties are due to the sequential publication of his equations that contain typographical errors; however, in later works he managed to correct these errors.

Förster summarized the experimental results of carbon dioxide uptake in the algae *Chlorella*: the reduction of a single molecule of carbon dioxide requires several photons which are absorbed by several chlorophyll molecules and the energy is transferred

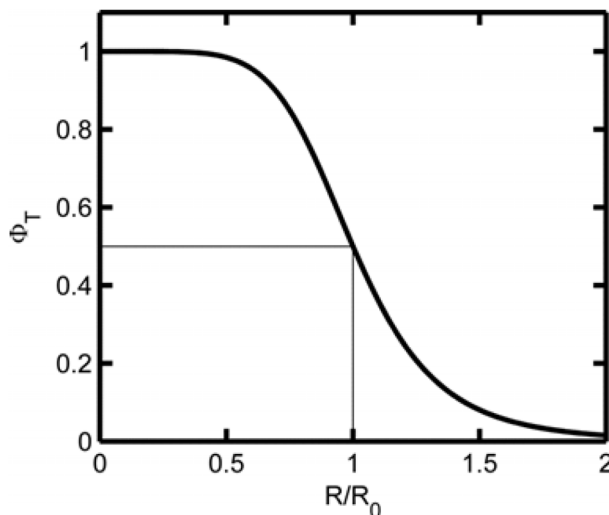


Fig. 2. A plot of the transfer efficiency versus R divided by R_0 .

to the photosynthetic unit where the carbon dioxide reduction occurs [Förster 1946]. Analysis of the effects of light intensity, reaction kinetics, and the lack of an induction period for the reaction are consistent with this model. However, as Förster cogently noted, there are other models, besides resonance energy transfer, that may be consistent with the experiments. He was aware of these processes, but his research focused on models of resonance energy transfer.

In the years prior to Foster's 1946 publication there were a series of papers published in the widely read *Journal of General Physiology* and also in the German journal *Naturwissenschaften* that reported on recent advances in the understanding of the mechanisms of photosynthesis [Emerson and Arnold 1932a,b; Emerson and Lewis 1942; Gaffron and Wohl 1936a,b]. Förster cited these papers in his later publications on his FRET theory and it is reasonable to assume that he was familiar with the interpretation of the experiments which gave credence to energy transfer mechanism in biological systems. The fact the Förster was aware of advances in the biology of photosynthesis is consistent with his broad interdisciplinary interests from chemistry to physics to biology.

Förster also cited some recent experiments on mutation and damage to chromosomes in the cell nucleus by X-rays or ultraviolet light. In these processes free radicals or electrons are produced during the radiation of biological specimens and that is the primary process. Then these particles migrate to distant sites and cause the secondary effects. The collision cross sections for the mutations and the damage are larger than the sites of the damage; this is consistent with energy migration from distant sites to the site of damage.

After Förster developed his approximate theory of resonance energy transfer in 1946 there appeared in the literature other papers that supported the mechanism of resonance energy transfer in photosynthesis. In 1947, for example there appeared a publication on the photochemical decomposition of carbon-monoxide myoglobin in which the decomposition occurs with the same quantum yield if the light is absorbed by the porphyrin component or the aromatic amino acids of the protein [Bücher and Kaspers 1947]. The authors stated that the reaction must occur in the porphyrin part of the molecule, therefore there must be energy migration from the aromatic amino acids to the porphyrin group.

Following these examples Förster stated that it is desirable to study the process of energy migration in molecules in order to understand the physical-chemical processes that occur in biological systems. He mentioned the processes in which the absorption of a quantum of light in one molecule causes changes in other molecules and gives the example of sensitized photochemical reactions in photography. In such processes, the energy is carried by a material particle, i.e. a liberated electron. The absorption of light with the liberation of electrons or excited atoms occurs in closely interacting molecules or in the gas phase over distances larger than molecules; but biological system present a problem of no or extremely low electrical conductivity. Förster posited that in biological systems energy migration occurs in the absence of material carries of the energy.

Next Förster summarized what is known about the phenomenon of *concentration depolarization* in which the fluorescence polarization of dilute viscous solutions of dye molecules decreased sharply with increasing concentrations of the dye. In the early 1920s experiments on the polarization of organic molecules such as Fluorescein, Rhodamine G, and Eosin, dissolved in gelatin or a viscous solution were significant because they represented the first observations of energy transfer in solution. These studies stimulated Jean Perrin to formulate his early theories of resonance energy transfer.

In addition to a series of experimental investigations that pointed to a possible role of resonance energy transfer in biological processes it is instructive for the reader to peruse two classics in the field of spectroscopy that most certainly were accessible to Förster. Peter Pringsheim's classic reference work, *Fluoreszenz und Phosphoreszenz im Lichte der neueren Atomtheorie, Dritte Auflage*, that covered the field up to 1928 and was most probably read by Förster [Pringsheim 1928]. In addition, Heinrich Gustav Johannes Kayser and Carl Runge edited the eight volumes of their seminal work *Handbuch der Spektroskopie* (Leipzig, 1900–1932); these classic volumes summarized the corpus of spectroscopic knowledge in that period [Kayser and Runge 1900–1932].

6.2.1 Fluorescence of organic molecules in solution and polarization phenomena

The term *fluorescence* was coined by George Gabriel Stokes [Stokes 1852]. Stokes showed that the fluorescence occurred at longer wavelengths than the excitation. The *Stokes shift* refers to this displacement of the wavelengths of the excitation and the emission and is the basis of all fluorescence spectroscopy because with the use of filters the very weak emission intensity can be separated from the relatively high intensity of the excitation light.

If polarized light (the electric vector of the electromagnetic field has a specific orientation) of the proper frequency interacts with an organic molecule whose transition dipole moment (represented by a vector of the same orientation as the electric vector of the illumination) the molecule will absorb a photon and undergo a transition from the ground state to the excited state. Then the molecule could radiate a photon and return to the ground state. That deactivation process is termed *fluorescence* if the emission is from the first excited singlet state of the molecule. If the lifetime of the fluorescence is less than the time for the molecule to rotate to a different orientation then the emitted fluorescence will also be polarized. However, if during the lifetime of the emission the molecule can rotate to a new orientation then the emitted light will have an electric vector that differs from that of the excitation light electric field vector orientation.

In 1920 Weigert discovered the polarization of fluorescent light from organic dyes dissolved in a viscous medium such as gelatin [Weigert 1920]. The polarization was observed because the viscous gelatin medium increased the rotational diffusion constant

of the molecule such that during the lifetime of the fluorescence the molecule was locked into a fixed orientation. Weigert observed that the measured polarization of the fluorescence increased with increasing viscosity of the medium.

Sergey Ivanovich Vavilov (1891–1951) was a Soviet physicist who is known for his studies of molecular luminescence. In 1934 Vavilov co-discovered the *Vavilov-Cherenkov effect*⁷. He also is known for the *Kasha-Vavilov rule*: the quantum yield of luminescence is independent of the excitation wavelength. There are exceptions to it.

Vavilov investigated the depolarization of fluorescence in solutions [Vavilov and Levshin 1923]. In 1923 Vavilov and Levshin studied the polarization of the fluorescence from many dyes that were dissolved in both water and in glycerol [Vavilov and Levshin 1923]. These authors correctly concluded that the loss of polarization was related to the rotation of the organic molecule during the fluorescent lifetime. Vavilov developed his theory of *inductive resonance* to explain the energy migration from one molecule to another molecule, and to explain the loss of polarization (*depolarization*) and the change in the lifetime of excited states during energy migration in solutions. Resonance energy transfer could occur between like molecules in a series of repetitive steps; the process of concentration depolarization of fluorescence in solution occurs with this mechanism [Vavilov 1943].

Besides energy migration in solution, Vavilov studied similar processes in condensed matter, i.e. in crystals that contain foreign atoms. He found that energy migration can occur over distances that are over one million times greater than the distances between molecules or atoms [Davydov 1962].

Other experimental observations supported the idea that there could be long-range energy transfer between molecules in solution [Gaviola and Pringsheim 1924]. Measurements of both the polarization and the intensity of the fluorescence of a solution of fluorescein in glycerol were made as a function of the concentration of the dye. As the concentration of the dye in the glycerol was increased the average distance between the dye molecules decreased. It was observed as the dye concentration increased, at a specific concentration of dye its polarization began to sharply decrease. This so-called *concentration depolarization* occurred at a dye concentration where the intensity of the fluorescence was still increasing and the average distance between molecules was in the order of 70 Å. This effect did not change the absorption spectra, the emission spectra, the quantum yield or the fluorescence lifetime which was the same as in very dilute solutions.

Within the fluorescent lifetime of the molecule the process of diffusion in the viscous glycerol could not account for the transfer of energy. Something strange was occurring: long-range energy transfer over distances greater than the sum of the collision cross-sectional radii was the cause of the reduced polarization.

An analysis by Gaviola and Pringsheim in 1924 invoked *Stokes rule* (the emission wavelength is longer than the absorption wavelength) which ruled out the trivial process of emission of a photon and its subsequent reabsorption as a mechanism for the observed depolarization [Gaviola and Pringsheim 1924]. The longer wavelength of the fluorescein fluorescence, due to the Stokes shift, could not be reabsorbed by the fluorescein. Therefore, they posited that a very small portion of the primary fluorescence would be reabsorbed and result in secondary fluorescence. These observations stimulated researchers to invoke new mechanisms of energy transfer between molecules close to each other and to develop quantitative theoretical models that contained experimental parameters that could be tested in experiments.

⁷ *Vavilov-Cherenkov effect* is radiation emitted when a charged particle traverses a dielectric medium at a speed greater than the phase velocity of light in that medium.

6.2.2 Assumptions of Förster's classical FRET theory

Förster initially invoked these assumptions in his two classical derivations of FRET [Förster 1946, 1951]: (1) D and A are modeled as two identical oscillators⁸, (2) there is very weak-coupling between D and A, which induces the resonance energy transfer. The interaction energy is much less than the energy of a single vibronic level⁹. Förster's other assumptions are: (3) a distribution of vibronic states in the first electronic state, (4) Coulombic coupling is approximated by dipole-dipole interaction between the donor D and the acceptor A¹⁰, (5) the acceptor A must have allowed electronic transitions; singlet-singlet transitions, (6) the degree of coupling is given by the spectroscopic overlap integral which is the overlap of the normalized emission spectrum of the donor D and the normalized absorption spectrum of the acceptor A. This is called *Förster's overlap integral* and is given the symbol J ¹¹, (7) dipole-dipole coupling is mediated by the dielectric constant¹², (8) the distance between D and A is denoted with the symbol R ¹³, (9) free rotation is assumed both for D and A¹⁴.

As Scholes explained, FRET is based on the very weak coupling. Implicit in that coupling are two assumptions: (1) the coupling to the bath is much greater than the coupling between the donor D and the acceptor A, and (2) the bath rapidly equilibrates following excitation of the donor D, and this equilibration is much faster than the rate of energy transfer. The excited molecules are in thermal equilibrium and relax to their lowest vibronic level of the first excited electronic state before resonance transfer occurs. The coupling between the donor D and the solvent is much stronger than the coupling between the donor D and the acceptor A involved in the energy transfer process. This condition results in the fact that FRET is both incoherent and irreversible [Scholes 2003].

6.3 Förster's classical derivation of FRET (1946)

Förster in his 1946 publication wrote that his model is "*eine einfache Überschlagsrechnung*" [a simple rough calculation]. He intended to present the assumptions and the

⁸ This assumption of exact resonance was modified by Förster in his probability argument in which exact resonance is only valid for a small fraction of the time of energy transfer.

⁹ The experimental verification of *very weak coupling* is the interaction does not alter the absorption and the emission spectrum of either molecule as compared to the spectrum of each molecule in the absence of the other molecule.

¹⁰ FRET assumes that to total electronic coupling is given by Coulombic coupling, and the Coulombic coupling is approximated by dipole-dipole coupling. The latter is based on a multipole expansion of the electric field in which only the second term (dipole) is retained. Muñoz-Losa et al. evaluated the validity and limitations of dipole-dipole coupling in FRET [Muñoz-Losa et al. 2009].

¹¹ It is measured from the absorption spectrum of the acceptor A, a plot of molar absorbance versus wavenumber or wavelength, and the overlap of the normalized emission spectrum of the donor D.

¹² Although it is usually taken as the value of the solvent, this may not be valid for all conditions; in particular it should be the dielectric constant of the matter through which the resonance energy transfer occurs. It could be the solvent or it could be a protein or a quantum dot. The symbol n refers to the refractive index of the medium through which the energy transfer occurs.

¹³ In the decades following Förster's FRET theory researchers have shown that multiple molecular configurations may require a statistical distribution for R [Grinvald et al. 1972; Haas et al. 1978; Haas and Steinberg 1984; Katchalski-Katzir et al. 1981].

¹⁴ Note that this assumption may not be universally valid.

derivation of a model for resonance energy transfer, which was based on the Perrins' previous work. Förster added a probability correction term to the Perrins' formulation which corrected the failure of the Perrins to derive the correct R_0 .

I use the modern notation to discuss the Förster's derivation¹⁵. Förster assumed, as did Perrin, that there are two identical molecules that can be considered as two identical oscillating dipoles; only the first oscillator is initially vibrating. The oscillating electric dipole moment is $\vec{\mu}(t)$ and $\vec{\mu}_0$ is the maximum value of the dipole moment. The angular frequency of the oscillation is ω .

$$\nu = \frac{\omega}{2\pi}$$

$$\vec{\mu}(t) = \vec{\mu}_0 \cos(\omega t).$$

An oscillating dipole will radiate energy and therefore lose energy with time [Jackson 1999]. This loss of energy can be described as:

$$E = E_0 e^{-k_{rad} t}$$

where the initial energy is E_0 and the rate of decay is k_{rad} .

The inverse of the rate of decay for the oscillating dipole to decrease its energy is a decay time τ :

$$\tau = \frac{1}{k_{rad}}.$$

The energy of each oscillating dipole is:

$$E = h\nu = \hbar\omega,$$

and the characteristic decay time¹⁶ of radiation [the mean duration of the emission] from the oscillating electric dipole τ is:

$$\tau = \frac{3\hbar c^3}{\vec{\mu}^2 \omega^3}.$$

The oscillating electric dipole from the donor D creates an electric field \vec{E}_D . This electric field interacts with the acceptor A, and the energy of interaction U is:

$$U = -\vec{\mu}_A \cdot \vec{E}_D.$$

The spatial distributions of the electric field from both a static electric dipole and that from an oscillating electric dipole are identical; therefore, Förster modeled the interaction energy U as the field from a static electric dipole. The energy of the interaction (dipole-dipole interaction) for two identical dipoles is:

$$U = \frac{\vec{\mu}^2}{R^3}.$$

During [one period of oscillation] the time of $1/\omega$, energy is transferred from one oscillator to the other due to coupling. For the case of resonance, these contributions

¹⁵ In his 1946 paper Förster wrote the following terms: c for the speed of light, \hbar for Planck's constant divided by 2π , U for the interaction energy between the two dipoles, M for the dipole moment, d for the separation distance between the two dipoles, d_0 for the critical separation distance for which the transfer time t_0 is equal to the excited state lifetime τ .

¹⁶ Förster cited a 1937 physics book, *Introduction to Theoretical Physics*, W. de Gruyter, p. 431, as the source of this equation.

add up, so that an energy $U\omega$ is transferred per unit of time. The transfer of the entire energy $\hbar\omega$ occurs in the time t_0 :

$$t_0 \sim \frac{\hbar}{U} \sim \frac{\hbar R^3}{\vec{\mu}^2}.$$

The transfer rate, k_T is the reciprocal of the time of transfer.

$$k_T \sim \frac{1}{t_0} \sim \frac{\vec{\mu}^2}{\hbar R^3}.$$

When R is equal to R_0 , the transfer time t_0 is equal to the lifetime τ :

$$t_0 = \tau$$

$$\frac{\hbar R^3}{\vec{\mu}^2} = \frac{3\hbar c^3}{\vec{\mu}^2 \omega^3}.$$

This yields for R_0 :

$$R_0 \sim \frac{c}{\omega} \sim \frac{\lambda}{2\pi}.$$

This theoretical value of R_0 is too large as compared to the experimental measurements. For a molecule that absorbs at 6000 Å, R_0 is 1000 Å. This is the same result as J. Perrin obtained. In his 1946 paper Förster omitted the refractive index and the orientation factor from his equations, but they appeared in his 1951 second classical derivation.

Förster cleverly pointed out that this discrepancy is due to the fact that up to this point the spectral lines were assumed to be very sharp, and the theory ignored the broadening of the spectral lines in solution at room temperature. Förster realized that the condition of exact resonance is not valid and results in an excessively large value for R_0 . The assumption of exact resonance is valid for only a small fraction of the time required for energy transfer. There is a low probability that the condition of exact resonance is valid through the energy transfer process. Förster arrived at this conclusion based on an assumption that typically the energy of interaction for very weak coupling between the donor D and the acceptor A is much smaller than their vibrational energies. Therefore, he had to modify his results by factors that correct for the presence of a range of excited states and he developed a *probability argument*.

The use of a *probability argument* is based on these assumptions: (1) the oscillators will have many cycles of oscillations prior to the transfer of energy and (2) their oscillation frequencies occur in a wide range, and (3) the excited states relax to their equilibrium states prior to energy transfer. Therefore, the probability of exact resonance is small. Förster's next step was to incorporate this low probability in his derivation.

In Figure 3, the absorption spectrum and the emission spectrum of a dye molecule in a solvent is plotted on the same figure and frequencies are angular frequencies. In the lower part of the figure is a simplified schematic of the same absorption and emission spectra. He considers the absorption and the emission spectra to be two rectangles of width Ω , the overlap of the two rectangles (the overlap of the absorption spectrum and the emission spectrum) is a strip of width Ω' .

Förster approximated the absorption and emission spectral lines as uniform distributions. He defined Ω as the spectral width of the absorption curve and the spectral width of the fluorescence curve since the oscillators are identical. Due to the Stokes' shift in frequency, there is a region of spectral overlap, defined as Ω' .

Förster calculated the probability w to be in resonance, as the probability for the two oscillators to overlap, multiplied by the probability that the two oscillators are

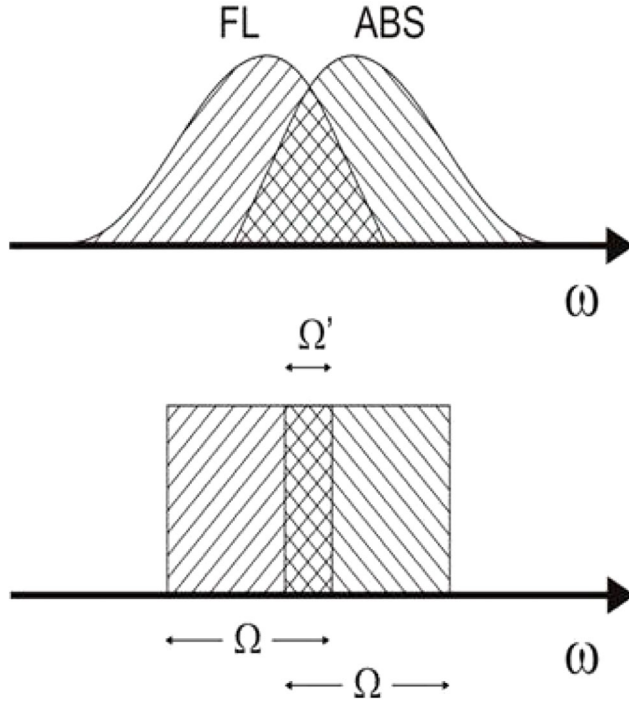


Fig. 3. Typical overlap of the intensity of the donor D emission and the absorption spectrum of the acceptor A, both on a frequency scale and normalized to 1 in intensity or absorption coefficient. The letter Ω is spectral width of the broadband emission spectrum and the broadband absorption spectrum and Ω' represents the frequency range of the spectral overlap. Frequencies are angular frequencies.

within the interaction energy frequency. The first term is the probability of spectral overlap is:

$$\frac{\Omega'}{\Omega}.$$

Förster wrote that the condition of resonance requires that the frequency of the one oscillator ω is within Ω' and at the same time the frequency of the other oscillator is identical to the frequency of the first oscillator within the frequency range:

$$\omega = \frac{U}{\hbar}.$$

The second probability term is:

$$\frac{U}{\hbar\Omega}.$$

Förster then calculated the total probability w , as the product of the first term and the second term:

$$w = \frac{\Omega'}{\Omega} \cdot \frac{U}{\hbar\Omega}$$

and by substituting the equation for U :

$$w = \frac{\Omega'}{\Omega} \cdot \frac{\bar{\mu}^2}{\hbar \cdot \Omega}.$$

Förster calculated the rate of energy transfer, k_T , and then multiplied that rate (for narrow line spectra of exact resonance) by the probability factor w , which corrected the expression to include the effects of a broadband spectra:

$$k_T = \frac{w}{t_0} = \frac{\bar{\mu}^2}{\hbar R^3} \cdot \frac{\bar{\mu}^2 \Omega'}{\hbar R^3 \Omega^2} = \frac{\bar{\mu}^4 \Omega'}{\hbar^2 R^6 \Omega^2}.$$

Förster included these corrective probability factors, which accounted for the probability that there is spectral overlap of the two oscillators and also, at the same time, the two oscillators are in exact resonance. Thus, he derived the rate of resonance energy transfer with a R^{-6} separation dependence between the donor D and the acceptor A molecules; this inverse six power of the separation distance is a seminal feature of FRET.

This formation does not allow the dipole moment, $\bar{\mu}$ to be explicitly evaluated; therefore, the energy transfer rate cannot be calculated. However, the Förster distance, R_0 , can be evaluated from the above equations with the following substitutions:

$$\tau = \frac{3\hbar c^3}{\bar{\mu}^2 \omega^3} \quad \text{and} \quad c = \frac{\lambda \omega}{2\pi}.$$

He then set the time, $t_0 = \tau$:

$$t_0 = \tau = \frac{\hbar^2 \Omega^2}{U^2 \Omega'} = \frac{\hbar^2 R_0^6 \Omega^2}{\bar{\mu}^4 \Omega'}.$$

In Förster's 1946 paper he wrote:

$$R_0 \sim \frac{\lambda}{2\pi} \sqrt[6]{\frac{\Omega'}{\tau_0 \cdot \Omega^2}}.$$

Förster then compared his calculated result with the experimental results. He wrote that for normal organic dye molecules, the width at half height of the emission is approximately 1/20 of the frequency, $\Omega \sim 1.5 \times 10^{14} \text{ sec}^{-1}$ for λ at 6000 Å. The ratio $\frac{\Omega'}{\Omega}$ which is the spectral overlap is approximately 0.10, and the lifetime is $\tau_0 = 0.5 \times 10^{-8} \text{ sec}$. The calculated R_0 is approximately 75 Å. Thus, Förster calculated for resonance energy transfer between two dye molecules separated by approximately 75 Å, the probability of resonance energy transfer is fifty percent. This is the correct order of magnitude for the critical distance.

6.4 Förster's second classical theory of FRET (1951)

In his book *Fluoreszenz Organischer Verbindungen* he developed a second classical theory of resonance energy transfer [Förster 1951]. In his book chapter Förster wrote equations that included the orientation factor κ , and the refractive index n of the media through which the resonance energy transfer occurs. Förster calculated the rate that a dipole acceptor A gains energy from the donor D oscillating dipole's *near field* radiation.

Förster initially assumed that the oscillators of the donor D and the acceptor A are in *exact resonance*. Then he evoked a spectral dispersion of both *oscillator strengths*, $f_e^D(\tilde{\nu})$ for the emission of the donor D, and $f_a^A(\tilde{\nu})$ for the absorption of the acceptor A, which corrected for the short times that the two oscillators are in resonance. The rate of resonance energy transfer from the donor D to the A is given by:

$$k_T = \frac{\kappa^2 c^4}{16\pi^2 n^4 m^2 R^6} \int_0^\infty f_e^D(\tilde{\nu}) \cdot f_a^A(\tilde{\nu}) \frac{d\tilde{\nu}}{\tilde{\nu}^2}$$

where m is the mass of the electron, and the other terms have been previously defined. He then expressed the rate of resonance energy transfer as:

$$k_T = \frac{9(\ln 10)\kappa^2 c^4}{128\pi^5 n^4 N' \tau_e R^6} \int_0^\infty f_e^D(\tilde{\nu}) \cdot \varepsilon^A(\tilde{\nu}) \frac{d\tilde{\nu}}{\tilde{\nu}^4}$$

where τ_e is the lifetime of the D and N' is Avogadro's number [Förster 1951]. Förster stated that this equation derived classically is identical with the equation that he derived in 1948 based on quantum mechanics.

7 Förster's 1948 quantum mechanical paper on FRET

In 1948 while Förster was still working in Göttingen he published his seminal paper *Zwischenmolekulare Energiewanderung und Fluoreszenz* and he acknowledged that his theory of energy transfer between molecules in solution is an extension of the earlier theories of Jean and Francis Perrin [Förster 1948]. As in his classical derivations of FRET, Förster's quantum mechanical derivation paper stated the mathematical steps and the assumptions that are the basis of his derivation, but the explanation of his reasoning for each step is either extremely sparse or nonexistent. This lack of explanation for his assumptions and mathematical steps presents difficulties in the reconstruction of his thinking behind the derivations.

Both the classical derivations and the quantum mechanical derivation of FRET resulted in the same equations for the rate of energy transfer k_T and the Förster radius R_0 ; but the mechanisms of the energy transfer are different. In the classical derivation of FRET the energy is progressively transferred between D and A; however, in the quantum mechanical derivation the energy transfer occurs at a random time and is instantaneous.

7.1 Förster's review of prior publications

Förster separated the cases of energy transfer in crystals with electronic conductivity (i.e., inorganic crystal phosphors and silver halide crystals in photographic emulsions) from those cases in which there is no electron conduction or migration but which exhibited energy migration. Förster discussed the application of his theory of resonance energy transfer to molecular crystals and to the *photosynthetic apparatus*. This is another example of his interdisciplinary knowledge from both chemistry and biology and his interest in bridging these fields.

Förster referred to the prior works on resonance energy transfer that were published in Germany, France, and the Soviet Union. He described the effect of *concentration depolarization* of fluorescence in solution, and concluded that this effect of a decrease with polarization with increasing concentration cannot be explained by the trivial process of sequential emission and reabsorption of photons [Gaviola and Pringsheim 1924]. Förster also cited several previous publications of Vavilov in the years of 1942, 1943 and 1944 on the subject of resonance energy migration. He cited the 1943 paper of S.I. Vavilov on *concentration depolarization of fluorescence* in which resonance energy transfer occurs in repetitive steps as an example of resonance energy transfer between like molecules [Feofilov and Sveshnikov 1940; Förster 1960a]. Förster noted that he only learned of the prior Vavilov publications from the years 1942 to 1944 after his 1946 publication on the classical theory of FRET.

7.2 Förster's assumptions and approximations in his quantum mechanical derivation

Förster presented the general ideas of his model: a solution of dye molecules dissolved in a solvent that does not absorb in the same spectral region as the dye molecules. He defined the rate of transfer as the number of transfers between pairs of molecules per unit time. In his model, the energy transfer is limited to simultaneous processes, and not the trivial case of successive emission and reabsorption. Förster postulated that the rate of energy transfer in his model will decrease rapidly with increasing distance of separation between the two molecules; therefore, the process of energy transfer would only occur during the lifetime of the excited state if the second molecule is present with a critical distance from the excited molecule.

Förster used Dirac's quantum mechanical theory to describe the transition between states [Dirac 1926, 1927]. Förster's FRET mechanism is based on the second-order perturbation theory of the electronic coupling between two molecules, the donor D and the acceptor A [Jammer 1989; Mehra and Rechenberg 2001].

FRET incorporated *Fermi's Golden Rule*¹⁷ which states that the rate of transition between the states R_{fi} from an initial ground state to a manifold or band of states is:

$$R_{fi} = \frac{2\pi |\vec{\mu}_{fi} \cdot \vec{E}|^2}{\hbar} \rho(\omega_{fi} = \omega)$$

where $\rho(\omega)$ is the density of states; the density of final states is evaluated at the frequency ω of the incident radiation, the electric dipole transition moment $\vec{\mu}_{fi}$, and the electric field strength is \vec{E} .

In terms of quantum mechanics the initial problem is to calculate the probability for the transition between two states: the first state of the system of two molecules has the molecule k in the excited state and the molecule l in the ground state, and the second state of the system has the molecule k in the ground state and the molecule l in the excited state.

Förster assumed, as did Jean Perrin previously, that the energy transfer is mediated by the Coulombic interaction between the two molecules, and that energy transfer "proceeds substantially more slowly than nuclear motion" [Förster 1948]. Förster called FRET the "theory of slow resonance transfer" [Förster 1960a]. The latter assumption permits the eigenfunctions of the molecules to be expressed in terms of the electronic coordinates, and the nuclear coordinates are suppressed in the treatment (Born-Oppenheimer approximation). These eigenfunctions are used to describe the stationary molecular vibrational states.

Dirac's theory gives the transition probability, defined in terms of the number of transitions per unit time for each molecule in the ground state [Dirac 1927]. The transition probability is the sum of the square of the matrix transition element for the interaction over all possible electronic-vibronic transitions.

Förster made the following definitions and additional assumptions. He assumed thermal equilibrium distribution over the vibrational levels of both molecules and the molecule interacts with the solvent and undergoes *vibrational relaxation*. He writes: "... it will be assumed that the in the initial state the energy of the molecular vibration has no definite value, but it changes rapidly during the excitation transfer". Therefore,

¹⁷ This eponymous rule is actually based on Dirac's 1926 work on time-dependent perturbation theory [Dirac 1926]. It took the name associated with Fermi because Fermi used it in his lectures on quantum mechanics that he gave at the University of Chicago. Fermi in the course of his lectures called it "*the golden rule*" and therefore it became associated with his name [Fermi 1955].

the molecule rapidly reaches the lowest vibrational level of a given electronic state after a transition. This process occurs *before* the process of resonance energy transfer. He assumed that the transitions occur from definite energy values for the initial states to a continuous manifold of final states.

The two molecules involved in the resonance energy transfer are k and l where no prime denotes the ground state (k) and a prime denotes the excited state (k'). The term F_{kl} is the probability for the transition from a state in which molecule k is excited and molecule l is in the ground state, into a different state, in which molecule k is in the ground state and molecule l is in the excited state. The stationary molecular vibrational states of the two molecules in these initial and final states are described by the quantum numbers: v'_k , v_l and v_k and v'_l . A prime signifies an excited state. The corresponding energies are w'_k , w_l , w_k , w'_l . The frequencies for the occurrence of individual values of the molecular vibration energy are given by two distribution functions, $g(w_l)$ and $g'(w'_k)$, which are normalized to 1 on an energy scale, a molecule in the ground and one in the excited state. W_0 is the energy difference between the lowest vibrational levels of the ground and the first excited electronic states.

With these assumptions, the rate of energy transfer, F_{kl} , expressed as the number of energy transfers per unit of time, is now expressed in terms of (1) the products of the two normalized distribution functions for the two molecules k and l and (2) the square of the magnitude of the matrix element for the interaction. The probability of the transition under the conditions of the conservation of energy is:

$$F_{kl} = \frac{2\pi}{\hbar} \int_{W=0}^{\infty} \int_{w_l=0}^{\infty} \int_{w'_k=0}^{\infty} g'(w'_k) \cdot g(w_l) \cdot [u_{kl}(w'_k, w_l; W_0 - W + w'_k, W - W_0 + w_l)]^2 \times dw'_k dw_l dW.$$

Förster then developed the interaction term and he assumed a critical condition. The molecules k and l , which could be large organic dye molecules, must satisfy the following separation condition: when the separation distance is much larger than the molecular dimensions of the molecules, then the energy of interaction reduces to the interaction energy between two dipoles. Therefore, he assumed that the interaction energy is given by the Coulomb interaction:

$$U(\vec{r}_k, \vec{r}_l) = -\frac{e^2}{n^2 |\vec{r}_k - \vec{r}_l|}$$

where the interaction energy, $U(\vec{r}_k, \vec{r}_l)$ is a function of the spatial coordinates of the electrons of molecule k and molecule l , the electronic charge is e , the refractive index of the solvent is n , and the dielectric constant is given as n^2 , where n is the refractive index of the medium through which resonance energy transfer occurs. The resulting matrix element for the interaction energy between two dipoles is:

$$U_{kl}(w'_k, w_l; w_k, w'_l) = \frac{1}{n^2 R_{kl}^5} \left[R_{kl}^2 \left(\vec{M}_k(w_k, w'_k) \cdot \vec{M}_l(w_l, w'_l) \right) \right] - 3 \left[\vec{M}_k(w_k, w'_k) \cdot \vec{R}_{kl} \right] \cdot \left[\vec{M}_l(w_l, w'_l) \cdot \vec{R}_{kl} \right].$$

This equation also indicates that the interaction energy between the molecules will depend on both their *relative orientation* and their *separation*. Förster defined the *matrix element of the transition moment* as:

$$\vec{M}_k(w_k, w'_k) = -e \int \phi_k(w_k, \vec{r}_k)^* \vec{r}_k \phi'_k(w'_k, \vec{r}_k) d\vec{r}_k.$$

The asterisk $*$ represents the complex conjugate of the quantity. The vector \vec{R}_{kl} is the vector between the corresponding molecular centers of gravity is selected as the origin of the electron position vectors \vec{r}_k . The first variable of \vec{M}_k denotes the nuclear vibrational energy in the electronic ground state, and the second variable denotes the nuclear vibrational energy in the electronic excited state.

For cases in which the energy transfer occurs more slowly than rotational Brownian motion, the rate of energy transfer must be averaged over all orientations of both molecules; this assumed that both molecules involved in the energy migration are freely rotating. For that case, Förster presented the average rate of energy transfer, which is expressed in terms of the minus-sixth power of the separation distance between molecules, the refractive index of the solution to the minus-fourth power and products of integrals that involve the square of the transition moment (based on dipole-dipole interaction energy) [Dirac 1927]. Again, following Dirac, the transition probability is given by the square of the matrix element of the interaction energy [Dirac 1927]. The resulting equation is:

$$\overline{|U_{kl}(w'_k, w_l; w_k, w'_l)|^2} = \frac{2}{3n^4 R_{kl}^6} \vec{M}_k^2 \vec{M}_l^2.$$

Förster (1965) defined U as the electronic interaction matrix element, which for the case of interest, dipole-dipole interaction, and allowed singlet-singlet transitions is:

$$U = \frac{1}{n^2 R_{ab}^3} \left[(m_a m_b) - \frac{3}{R^2} (m_a R_{ab}) \cdot (m_b R_{ab}) \right]$$

$$U^2 = \frac{\kappa^2 |m_a|^2 |m_b|^2}{n^4 R_{ab}^6},$$

where m_a, m_b are the *electronic transition moments* between the ground and the excited state, of molecule a and molecule b . Förster then introduced the *Boltzmann factors* $g'(E)$ and $g(E)$ for the excited molecule and for the unexcited molecule and obtained:

$$k_T = \frac{\kappa^2}{n^4 \hbar^2 R_{ab}^6} \int \left[m_a^2 \int g'(E'_a) S_a^2(E'_a, E'_a - h\nu) dE'_a \right]$$

$$\times \left[m_b^2 \int g(E_b) S_b^2(E_b, E_b + h\nu) dE_b \right] d\nu.$$

The factors S_a^2 and S_b^2 are the *Franck-Condon factors*. Each of the terms with the square brackets under the integral sign are related to the spectroscopic transition probabilities between the ground and the excited states of molecules a and b . Within each square bracket are the following terms: the square of the electronic transition moment and a *Franck-Condon factor* that are averaged over the Boltzmann distribution of the original state. For example, the first square bracket is proportional to the spectral density of the emission spectrum of molecule a for thermal equilibrium in its excited state. The second square bracket is proportional to the spectral density of the absorption spectrum of molecule b .

Now comes the most important point: “therefore, the integral shown above with the two square brackets is proportional to the overlap integral of the fluorescence spectrum of a and the absorption spectrum of b ”. Therefore we can calculate quantitatively the rate of resonance energy transfer between molecules a and b from spectral data. This is developed in Section 7.3 into his phenomenological theory of FRET.

Förster’s model of resonance energy transfer permits transfer between molecules of the same or of different kinds provided that for the second case (D and A are

different) the excited state of the acceptor A is of lower energy than the excited state of the donor D. Finally, if the spectra are represented on a wave number scale ν and the fluorescence spectrum is normalized the rate of resonance energy transfer is:

$$k_T = \frac{9000 (\ln 10) \kappa^2 \Phi_D^0}{128 \pi^5 n^4 N \tau R^6} \int f_a(\tilde{\nu}) \varepsilon_b(\tilde{\nu}) \frac{d\tilde{\nu}}{\tilde{\nu}^4}$$

where Φ_D^0 is the quantum yield of the donor in the absence of the acceptor, n is the refractive index of the medium through which the energy transfer occurs, N is Avogadro's number, R is the distance between the donor and the acceptor, τ is the lifetime of the donor in the absence of the acceptor, $f_a(\tilde{\nu}) d\tilde{\nu}$ is the normalized fluorescence of the donor in the wavenumber range of $\tilde{\nu}$ to $\tilde{\nu} + d\tilde{\nu}$, $\varepsilon_b(\tilde{\nu})$ is the absorption coefficient of the acceptor at the wave number $\tilde{\nu}$, and the other terms have been previously defined. The term $\varepsilon(\nu)$ is the molar decadic extinction coefficient and $f(\nu)$ is the fluorescence spectrum, normalized to unity on a frequency scale. He stated that the transfer rate of RET is related to the spectral properties by the following equations:

$$\varepsilon(\nu) = \frac{2^2 \pi^2 N m^2 \nu}{3 (\ln 10) n \hbar c} \int g(E) S^2(E, E + h\nu) dE$$

$$f(\nu) = \frac{2^5 \pi^3 n \tau_e m^2 \nu^3}{3 \hbar c^3} \int g'(E') S^2(E', E' - h\nu) dE'.$$

The notation is: m is the electronic transition moments of molecule, $\varepsilon(\nu)$ is the molar decadic extinction coefficient and $f(\nu)$ is the fluorescence quantum spectrum, normalized to unity on a frequency scale. The symbol N is the number of molecules per millimole, c is the velocity of light, and τ_e is the intrinsic fluorescence lifetime, in the absence of energy transfer (FRET). Again, he states that an appreciable overlap of the spectra is a requirement for resonance energy transfer under very weak coupling.

7.3 Förster links his equations for transition probabilities with spectra

Förster made a seminal advance with his crossover from the theoretical equations of Dirac's quantum mechanics to the experimentally accessible quantities such as absorption and emission spectra and excited state lifetimes. It was his astute realization that some of the terms in the quantum mechanical theory of FRET; i.e. the expression for the rate of energy transfer can be linked to spectral properties of the donor D and the acceptor A. This led to a *phenomenological* approach that was the first major step to place this technique from the hands of spectroscopists to the hands of biochemists and biologists [Gottfried and Yan 2004]. Perhaps his unique development of FRET was that he connected the rate constant for FRET with parameters that could be obtained from spectroscopic measurements of the donor D and the acceptor A.

Förster showed that the transition probability averaged over all orientations for the donor D and the acceptor A contains a product of two terms, the first of which is related to the fluorescence spectrum and the second term is related to the absorption spectrum. Förster stated that the absorption spectrum of a molecule can be more easily measured than the fluorescence spectrum; therefore, he wrote that is convenient to also relate the first term of the product to the absorption spectrum. For this assumption he reasoned that in the polyatomic molecules that he is considered (fluorescent dye molecules) the electronic excitation energy is distributed over many atoms in the molecule, and the vibrational degrees of freedom are similar in both the excited state and the ground state. Therefore, he assumed that the transition moment is an approximately symmetric function of the vibrational energy in the ground

and in the excited states. He then explained that in light of the above argument the distribution functions of the ground and the excited states are approximately equal. These arguments established the similarities of the fluorescence spectrum and the absorption spectrum as being mirror images. Additionally, he invoked *Levshin's law of mirror correspondence* [Levshin 1931a,b]¹⁸.

Förster assumed the validity of the *mirror law of fluorescence* and the fact that the overlap of the absorption and fluorescence spectra occurs only in a narrow region. Förster then defined R_0 as the critical molecular separation, below which there is energy transfer during the excited state lifetime.

Both Förster's classical derivation and his quantum mechanical derivation yielded the identical equations for both the energy transfer rate constant for k_T .

The rate of energy transfer is:

$$k_T = \frac{1}{\tau_D^0} \left(\frac{R_0}{R} \right)^6.$$

The Förster radius is given below. As pointed out by Braslavsky et al. the units in the equations for the Förster radius must be given explicitly [Braslavsky et al. 2008].

$$\begin{aligned} \left(\frac{R_0}{\text{cm}} \right)^6 &= \frac{9000(\ln 10)}{128\pi^5} \left(\frac{\text{mol}^{-1}}{N_A} \right) \times \left(\frac{\kappa^2 \Phi_D^0}{n^4} \right) \left(\frac{J^\lambda}{\text{mol}^{-1} \text{dm}^3 \text{cm}^3} \right) \\ \left(\frac{R_0}{\text{cm}} \right)^6 &= \frac{9(\ln 10)}{128\pi^5} \left(\frac{\text{mmol}^{-1}}{N_A} \right) \times \left(\frac{\kappa^2 \Phi_D^0}{n^4} \right) \left(\frac{J^\lambda}{\text{mol}^{-1} \text{dm}^3 \text{cm}^3} \right). \end{aligned}$$

The degree of spectral overlap between the emission of the donor D and the absorption of the acceptor A is expressed as the *spectral overlap integral* $J(\lambda)$ is:

$$J(\lambda) = \frac{\int_0^\infty F_D(\lambda) \varepsilon_A(\lambda) \lambda^4 d\lambda}{\int_0^\infty F_D(\lambda) d\lambda}$$

where $F_D(\lambda)$ is the donor fluorescence per unit wavelength interval, $\varepsilon_A(\lambda)$ is the molar extinction coefficient of the acceptor at the wavelength λ , the quantum yield of the donor D in the absence of the acceptor A is Φ_D^0 , the refractive index of the medium is n , Avogadro's number is N , and the lifetime of the donor D in the absence of the acceptor A is τ_D^0 .

The importance of Φ_D^0 is that there are many pathways to deactivate the excited state of the donor D; they all compete with each other to deactivate the excited state of the donor D. The terms inside of the integral are: $F_D(\lambda)$ is the corrected fluorescence intensity of the donor D in the wavelength range λ to $\lambda + \Delta\lambda$ under the condition that the total intensity is normalized to unity; $\varepsilon_A(\lambda)$ is the extinction coefficient of the acceptor A at the wavelength λ .

The last two sections of Förster's 1948 paper deal with energy transfer in solution, and a discussion of concentration depolarization. In this section Förster takes the rate of resonance energy transfer to be dependent on the inverse sixth power of the separation distance between molecules in the interaction term and calculates the transfer for an assumed *statistical distribution* of dye molecules in solution.

¹⁸ The *Levshin mirror law of fluorescence* states that there is an approximate mirror symmetry in the intensity patterns of both the absorption and fluorescence spectra.

It is important to point out that in solution there is a distribution of separation distances, and the calculation proceeds by averaging over this distribution. Using another approach Förster makes the assumption that the dye molecules are positioned on a three-dimensional lattice: simple cubic, face-centered cubic, body-centered cubic, and the diamond lattice. This model leads to the transfer of energy from molecule to molecule to be described by a differential equation similar to that used for heat conduction and diffusion.

Then he described concentration depolarization. Förster's analysis assumed that the interaction energy can be replaced by its average over all orientations of the relative dipoles at a fixed distance. Therefore, he stated that his theory is only correct for low viscosity solutions of dye molecules in which the Brownian rotational diffusion of the molecules is much more rapid than the process of energy transfer. Förster stated that an exact theory would have to incorporate the relative orientations of the molecular transition moments.

At very high concentration of dye molecules in solution, the molecular separation is a function of the size of the molecules. Now the interaction can no longer be correctly described by dipole-dipole interactions, and Förster's concentration depolarization theory of resonance energy transfer is no longer valid. Also, the previous assumption of thermal equilibrium of vibrational motion may no longer be valid. Instead of the very weak dipole-dipole interactions of the Förster theory, when energy transfer is mediated by strong interactions, a new and different theoretical model is required.

Such strong interaction models of energy transfer are based on quantum mechanical treatment of the entire assembly of molecules and were formulated by others [Frenkel 1931a,b, 1950; Peierls 1932; Franck and Teller 1938]. These models are very different from the very weak coupling model of FRET.

At the end of this historic paper Förster notes that chlorophyll in the photosynthetic apparatus and biological molecules in general may be ordered into some crystal lattice or similar array, and therefore other theoretical models, differing from the Förster theoretical model, such as the Frenkel exciton model, may be appropriate to describe energy transfer in these biological cases [Frenkel 1931a,b, 1950].

There is a clear demarcation between the Förster's FRET and all the previous works. In all the previous works the authors *assumed exact resonance*, and that the *spectroscopic bands are extremely sharp*. Förster was the first to look at the interaction of the electronic systems with the molecular vibrations. This broadens the very sharp electronic frequency into a broader frequency range. In solution a redistribution between the vibrational levels occurs due to thermal relaxation. This accounts for the important *Stokes shift* between the absorption spectrum and the emission spectrum. These effects result in resonance conditions which are completely different from the idealized very sharp spectroscopic bands of ideal systems [Förster 1960a].

7.4 Förster's post 1948 publications

Förster's 1959 *Spiers Memorial Lecture on Transfer Mechanisms of Electronic Excitation* elucidated some of the arguments used to rule out the case of photon emission and reabsorption in the discussion of nonradiative energy transfer and provided details on the mechanism of FRET [Förster 1959]. Förster described how to identify the various energy transfer mechanisms: non-trivial transfer, emission and reabsorption of a photon, formation of an intermediate complex, and a collision. For the non-trivial nonradiative energy transfer the following properties are observed: no dependence on volume, no dependence on viscosity, a decreased lifetime of the donor D, and both absorption and the emission spectra of the donor D are unchanged. The latter is a characteristic of the very weak coupling of FRET.

Next he stated that it is unlikely that long-range nonradiative energy transfer can occur within the short lifetimes of excited state molecules; but the very weak coupling may be sufficient for FRET if a *resonance condition* is fulfilled. He explains the *resonance condition* as follows. The donor D absorbs a photon and is excited to a high vibrational level of the first singlet electronic state; it thermally relaxes to the lowest vibrational level of the first singlet state and is consistent with the *Franck-Condon principle*. The energy levels of the acceptor A match the energy levels of the donor D and that is the *condition of resonance*. The experimental feature of this resonance condition occurs when the broad spectra of polyatomic molecules in solution show an overlap between the absorption spectrum of the acceptor A and the fluorescence spectrum of the donor D. He denoted this energy transfer mechanism either *resonance transfer* or *transfer by inductive resonance*. During resonance transfer the donor D in an excited state will become deactivated and *simultaneously* the acceptor A that was in the ground state will be in the excited state. The transfer occurs before the emission of the donor D occurs, and the transfer can only occur over limited distances. The coupling of the two molecules is strongest for *allowed* electric-dipole transitions, but Förster explained that symmetry forbidden transitions can become partially allowed by combination with specific molecular vibrations, or with intercombination transitions that can occur by the mixing of states of different multiplicities.

Förster then defined R_0 , given in the last section, and noted: “this is valid for any *thermal equilibrium distribution* over the vibrational levels of both molecules, provided that the spectra are taken at the corresponding temperature . . . R_0 increases with the quantum yield of the sensitizer [D] and with the overlap of the spectra. In typical cases, R_0 values of 50 Å to 100 Å have been calculated” [Förster 1959].

Förster described the work of Dexter and stated that at small separation distances exchange terms in the interaction must be considered [Dexter 1953]. This restriction at small distances is often ignored [Beljonne et al. 2009; Scholes 2003]. Förster stated there are several mechanisms of energy transfer. At high concentrations of dye molecules in solution there is the formation of dimers with different spectra from the monomers. The analysis of energy transfer in these dimers requires mechanisms that are different from FRET [Davydov 1962; Cruzeiro-Hansson and Takeno 1997; Kasha 1959, 1963, 1991]¹⁹.

In 1960 Förster was a Professor in the Technischen Hochschule, Stuttgart, and head of the Laboratorium für Physikalische Chemie und Elektrochemie. *Transfer Mechanisms of Electronic Excitation Energy* was published in *Radiation Research Supplement* [Förster 1960a]. Förster discussed experiments on energy transfer in biological systems, possible energy transfer mechanisms, the general principles of resonance energy transfer, theory of slow resonance transfer and resonance energy transfer in proteins. This paper is further evidence of his long term interest in energy transfer in biological systems and his interdisciplinary (physics, chemistry, and biology) approach to research. At the end of his paper Förster concluded: “. . . resonance transfer of excitation is able to explain, in principle, the different photochemical and fluorescence experiments with proteins and photosynthetic systems” [Förster 1960a].

In 1960 Förster, Kallmann, and Kasha participated in international conference on the *Comparative Effects of Radiation* [Förster 1960b]. This conference included discussions of their respective papers following their presentations. Förster’s chapter in the book that is based on the conference papers and discussions contains some insights to his 1960 concepts of resonance energy transfer. This is fourteen years after he first

¹⁹ In 1962 Michael Kasha and Max Oppenheimer, Jr. translated the classic book of Davydov, *Theory of Molecular Excitons*, from Russian to English [Davydov 1962]. This book also lists the complete publications of A.S. Davydov as well as a supplementary biography of publications on excitons and it an important resource.

published his classical theory of resonance energy transfer. From a pedagogical perspective in this paper, which stressed the quantum mechanical aspects of FRET and compared it with alternative mechanisms of energy transfer, Förster is significantly clearer in his description FRET than in his earlier papers. In 1967 Förster published a chapter that stressed the novel spectroscopic aspects of excitation transfer and is discussed in Section 7.5 [Förster 1967].

Over a period of years Förster’s publications propagated typos in his equations; some of them were corrected in his later publications. In some cases I corrected the equation by substituting the term \hbar^2 when the original German text erroneously contained the term \hbar [Förster 1946]. Additionally, Förster incorrectly wrote π^6 in his 1946, 1959, and 1960 papers and his 1951 famous book, and this error was corrected to π^5 in 1965 [Förster 1951, 1959, 1960a,b 1965].

8 Classification of excitation transfer in terms of coupling strength

The role of the coupling strength in the evolution of schemes to classify excitation transfer originated with the seminal collaborative work of Franck and Teller [Franck and Teller 1938]. Förster explicitly stated that all later work on excitation transfer can be traced back to 1938 paper of Franck and Teller [Förster 1960b]. That is an important historical point, at least in the words of Förster, on the paths to the development of excitation transfer mechanisms. Förster follows the same classification for the strengths of the coupling between the donor and the acceptor that was previously used in 1838 by Franck and Teller in their study of migration and photochemical action of excitation energy in crystals. Franck and Teller begin their paper with the following question: “to what extent can excitation energy absorbed by an arbitrary cell of the crystal be used photochemically at a specific point which may be far removed from the absorbing cell”? Their theoretical analysis is then applied to the behavior of polymerized pseudisocyanines, to the photosynthetic unit, and to the theory of sensitized photographic plates.

In 1957 Simpson and Peterson published a paper in which they defined the criteria for strong coupling, intermediate coupling, and weak coupling in various mechanisms of resonance energy transfer in van der Waals solids [Simpson and Peterson 1957]. They acknowledged the prior work [Franck and Teller 1938].

Förster developed his scheme of classification of energy transfer in terms of coupling strength by following the prior publications which he carefully acknowledged. The *Simpson and Peterson criterion* was discussed and cited by Förster in his 1960 book chapter on excitation transfer and in his 1965 book chapter on delocalized excitation and excitation transfer [Förster 1960b, 1965, 1967]. Förster and later Kasha used their criterion in discussing their classification of energy transfer [Förster 1960b; Kasha 1959, 1963, 1991].

The *Simpson and Peterson criterion* is the ratio of the energy of interaction between two molecules which is denoted by the symbol U , to the vibronic bandwidth which is denoted by the symbol $\Delta\varepsilon$.

The strong coupling case is defined as:

$$\frac{U}{\Delta\varepsilon} \gg 1.$$

The intermediate coupling case is defined as:

$$\frac{U}{\Delta\varepsilon} < 1.$$

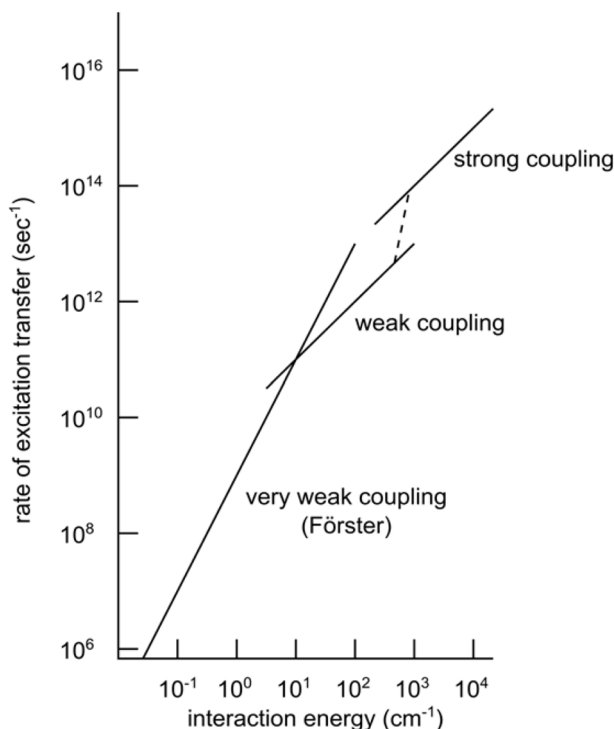


Fig. 4. A log-log plot of the rate of excitation transfer (transfers/sec) versus the interaction energy (cm^{-1}). The plot is modified from [Förster 1960b; Kasha 1991]. The broken line represents the intermediate case.

The weak coupling case is defined as:

$$\frac{U}{\Delta\varepsilon} \ll 1.$$

The next part of Förster's paper is a discussion of the theory of several mechanisms of energy transfer [Förster 1960b]. Two molecules can transfer energy in a nonradiative process if their transition energies from the ground state to the first excited state are approximately equal for both molecules or if they are in resonance with each other. That is the source of the name *resonance energy transfer* or *transfer by inductive resonance*. The magnitude of the interaction energy U determines the sharpness of this resonance. The molecular parameters such as the *Franck-Condon energy*, ΔE which is the energy difference between the nuclear equilibrium structures of the ground and the excited states, and the *Stokes shift* between the absorption and the emission spectra are important.

Förster's 1967 description of the three classes of coupling based on the strength of their interaction [Förster 1967]. Figure 4 is a log-log plot of the rate of excitation transfer (transfers/sec) versus the interaction energy (cm^{-1}). The log-log plot shows the functional relationships for strong coupling (Simpson and Peterson) with a slope of R^{-3} , weak coupling (Simpson and Peterson) with a slope of R^{-3} , and very weak coupling or vibrational-relaxation resonance transfer (FRET) with a slope of R^{-6} .

For the case of *strong coupling* all of the vibronic sublevels levels of the donor are in resonance with those of the acceptor. The case of *strong coupling* has the interaction energy, between the two molecules that undergo resonance energy transfer, very much larger than the vibronic bandwidths of each molecule. For strongly coupled systems

there are large differences between their absorption spectra and those of their components. For two molecules in resonance with each other, with their excitation energies differing by less than the interaction energy U , the energy transfer rate constant is approximately:

$$k_T \approx \frac{4|U|}{h}$$

where h is Planck's constant. For the case of *strong coupling*, when the interaction energy can be approximated by a dipole-dipole interaction, then the rate of transfer is proportional to R^{-3} , the inverse third power of the separation distance between the two molecules.

For the case of *weak coupling* the transfer rate is slower than for the previous case. The nuclei of donor in the excited state undergo several vibrations around their equilibrium positions before energy transfer occurs. For the case of weak coupling, the interaction energy is significantly smaller than the absorption bandwidth, the energy from the ground state of the donor to the first electronic state, and the interaction energy is much less than the energy bandwidth of a single vibronic energy level.

For the case of *weak coupling* there are small changes in the absorption spectrum. After excitation, the nuclei undergo several vibrations about their new equilibrium position *before* energy transfer occurs. The excitation transfer occurs over several vibrational periods and is associated with both a readjustment of the nuclear equilibrium positions and a transfer of vibrational excitation. Since the interaction energy is much less than the energy of the vibronic sublevels, resonance can only exist between the individual vibronic levels of both molecules; therefore, only *pairs* of vibronic transitions are in resonance with each other. Weak coupling contrasts with the case of strong coupling: the rate of energy transfer is faster than vibronic relaxation, but slower than the motion of the nuclei. The rate of energy transfer is slower than for the case of strong coupling. The energy transfer rate constant is approximately:

$$k_T \approx \frac{4|U| S_{\nu,\nu'}^2}{h}$$

where U is the interaction energy between the two molecules, h is Planck's constant, and $S_{\nu,\nu'}$ can be considered as the overlap integral between the vibronic states ν and ν' . Note that $S_{\nu,\nu'} < 1$, is the Franck-Condon integral of the intramolecular transition. The product $US_{\nu,\nu'}^2$ is the interaction energy between the vibronic transitions.

For the case of *weak coupling*, the rate of transfer is proportional to R^{-3} , the inverse third power of the separation distance between the two molecules.

The case of *very weak coupling*, which occurs in FRET, is different. For the case of *very weak coupling* the interaction energy is much less than the vibrational band width, and therefore only very small regions of the corresponding bands are in resonance with each other. This restrictive condition is somewhat mitigated by the fact that both thermal and solvent effects result in vibronic bandwidths that are broadened. This more severe requirement for resonance introduces an additional factor into the rate constant for energy transfer:

$$\frac{S_{\nu,\nu'}^2}{\Delta\varepsilon}.$$

For very weak coupling the rate of energy transfer between the two molecules is approximately:

$$k_T \approx \frac{4\pi^2 (US_{\nu,\nu'}^2)^2}{h\Delta\varepsilon} = \frac{4\pi^2 U^2 S_{\nu,\nu'}^4}{h\Delta\varepsilon}.$$

For Förster's FRET the rate of energy transfer is proportional to the square of the interaction energy between the two molecules, to the square of the overlap integral

of the vibrational interactions. For *very weak coupling*, the rate of resonance energy transfer is proportional to R^{-6} .

In the case of *very weak coupling*, the redistribution of vibrational energy occurs more rapidly than the resonance excitation transfer. There is no oscillation of energy transfer between the donor and the acceptor. A key characteristic of very weak coupling is that the absorption spectra of the donor D in the presence of FRET is unchanged. The explanation for this is that the vibrational relaxation takes place prior to the process of resonance energy transfer. A requirement for excitation transfer under very-weak coupling is an appreciable overlap of the spectra of the donor D and the acceptor A. This mechanism permits energy transfer between molecules of the same kind and also between different kinds of molecules. For the latter process, the energy of the excited state of the acceptor A is slightly lower than the energy of the excited state of the donor D.

9 Experimental tests of FRET and their role in the dissemination of FRET

Typically a physical theory is subjected to experimental validation before it is accepted by the mainstream science community. For FRET this was accomplished, to very different degrees, by two independent groups who published their papers within two years of each other.

The first paper had the following title: *Energy Transfer. A System with Relatively Fixed Donor-Acceptor Separation* [Latt et al. 1965]. To the typical reader, who is not a specialist in molecular spectroscopy, the two words energy transfer would not indicate that this paper validates an optical technique that could be useful to the measurement of changes in binding or molecular configuration on the nanometer scale. The paper appeared in the *Journal of the American Chemical Society*; a publication known for its high quality papers, but not typically read by the biological community.

I focus on the stereochemistry of the experimental design as developed in the Blout group at Harvard Medical School, Boston, MA. The requirements to test the validity of the inverse sixth power law are as follows: the synthesis of a rigid molecule with two sites separated by a known distance onto which a donor D chemical group could be covalently attached to one site and an acceptor A chemical group could be covalently attached to a second site. The average D-A separation was obtained from *Dreiding molecular models*, but although the structure of the “bisteroid” molecule is planar and rigid there was a slight uncertainty in the calculated separation distance due to the possible rotation of the donor D and the acceptor A groups around the bonds at the covalent attachment sites.

The test procedure was as follows. The average D-A distances measured from molecular models and assuming free rotation of the donor D and the acceptor A around the chemical bonds at the attachment sites was compared with the calculated distances from the Förster’s FRET. The experimental distances agreed with the distances obtained from the models. The rate of resonance energy transfer was a function of the inverse sixth power of the separation distance between the donor D and the acceptor A groups. The authors wrote: “... thus is a potentially powerful tool for investigating interactions between certain molecular groups and changes in the microstructure of macromolecules”, but this conclusion was not widely known in the biological community.

A paper with the title *Energy Transfer: A Spectroscopic Ruler* was published in *Proc. Natl. Acad. Sci. USA*, a journal that was more accessible to the biological community, placed FRET in the limelight as a distance measurement tool [Stryer and Haugland 1967].

The authors set out to test the dependence of the rate of energy transfer on R . Their experimental protocol had two requirements: first, to synthesize a set of oligomers of poly-L-proline which acted as spacers between the D-A pairs; and second, to confirm that the sets of oligomers behaved as rigid rods in solution. The reason for the second point is that for oligomers that are flexible, the D-A pairs would not be separated by a fixed distance but would be a statistical average of varying distances. α he authors used measurements of optical rotatory dispersion (ORD) which measures the optical rotation of the plane of polarized light when it traverses a solution containing the oligomers. From these measurements they determined that the oligomers with the numbers 5 to 12 poly-L-proline spacers between the donor D and the acceptor A molecules were rigid rods. Furthermore, the authors showed that the emission of the fluorescence of the molecules was completely depolarized, and they concluded that the angular relationship between the donor D and the acceptor A transition moments is randomized during the excited state lifetime. They estimated R for each oligomer from molecular models. Their calculated results were in excellent agreement with FRET. The authors then concluded: "these results suggest that under suitable conditions the energy transfer process can serve as a *spectroscopic ruler* in the 10-to-60 Å range". In a prescient statement Stryer and Haugland wrote: "a particularly interesting possibility is that energy-donor-acceptor pairs might be used to reveal proximity relationships in biological macromolecules".

The impact of each of these two papers on the community of chemists and biologists who are not spectroscopists may be estimated by seeking the total number of citation for each of these papers. I accessed the *Web of Knowledge* on the internet to determine the total number of citations for each paper from its year of publication to September 3, 2013. The number of total citations for the Latt, Cheung, Blout 1965 paper is 161. The number of total citations for the Stryer, Haugland 1967 paper is 1061. My posited explanation for this large disparity is twofold. First, the Latt et al. paper was published in a journal that was not typically read by researchers in the biology community. Second, the Stryer and Haugland paper was published in a journal that was read by biologists, and more over the title contained the words "*A spectroscopic ruler*", which focused attention on the important potential application of this optical technique. While both papers ended with the statement that energy transfer could be used to investigate interactions between certain molecular groups I posit that the wording of the title and the concluding sentence of the Stryer and Haugland paper caught the attention of the biological community²⁰.

10 Arnold and Oppenheimer's 1950 publication on internal conversion in photosynthesis and its similarity to FRET

Arnold, a biophysicist, and Oppenheimer, a theoretical physicist, collaborated on the problem of energy transfer mechanisms in photosynthesis prior to the Second World War that resulted in a published abstract with the title: "*Internal conversion in photosynthesis*" [Oppenheimer 1941]. After the war they published their completed theory: "Internal conversion in the photosynthetic mechanism of blue-green algae" [Arnold and Oppenheimer 1950].

²⁰ Two years later there appeared a publication that validated the proportionality of the kinetics of singlet-singlet energy transfer to Förster's *overlap integral* [Haugland, Yguerabide, Stryer 1969]. This publication in the *Proceedings of the National Academy of Sciences* could have also helped to promote Förster's FRET theory to an interdisciplinary group of readers that included members of the biochemistry and the biology research communities.

I have no knowledge if Förster was aware of the publication of Oppenheimer's abstract in 1941 in the *Physical Review*; he certainly did not cite it in his 1946 publication on FRET. However, the Arnold and Oppenheimer paper of 1950 cited Förster's 1946 publication on his classical theory of resonance energy transfer [Förster 1946]. I wonder if Arnold and Oppenheimer's collaboration was not interrupted by the Second World War whether they would have developed a general theory of resonance energy transfer that preceded Förster's 1946 paper?

What is remarkable is that Oppenheimer was able to use his knowledge of *internal conversion*²¹ that occurs in radioactive elements, and which is a known mechanism in nuclear physics and apply an analogous mechanism to the problem of energy transfer in photosynthesis [Arnold and Oppenheimer 1950].

Previously researchers discovered that light is absorbed by dyes which are called accessory pigments. Chlorophyll does not absorb these wavelengths. The paradox is that the energy of the absorbed light must be transferred from the accessory pigments to the chlorophyll molecules in order to facilitate photosynthesis. Arnold and Oppenheimer worked to develop a mechanism for this energy transfer that successively occurs over several molecules. In their paper we read: "this new process is, except for scale, identical with the process of *internal conversion* that we have in the study of radioactivity" [Arnold and Oppenheimer 1950].

Arnold and Oppenheimer posited that the highly efficient energy transfer between chlorophyll molecules is not due to repetitive light emission and subsequent light absorption. They calculated that if the chlorophyll molecules were separated by distances that are much smaller than the wavelength of the chlorophyll fluorescence then the intermolecular energy transfer would be greatly enhanced. They derived an equation for the ratio of the number of quanta of energy that are transferred and the number of quanta that are emitted in fluorescence.

First, they studied the quantum yield of chlorophyll and phycocyanin in the plant *Chlorella*. They concluded that any energy transfer mechanism must have a high efficiency in the neighborhood of 90 percent. Second, they listed three possible mechanisms for the purported high efficiency energy transfer: (1) collisions of the second kind in which the collision transfers the energy between two molecules, (2) the emission of energy from one molecule and its subsequent absorption by a second molecule, and (3) the process of *internal conversion*, or the resonance transfer of energy from one oscillator to another in resonance with it. The authors then evaluated these three possibilities. The first option of collisions is not dependent on the existence of resonance between the two molecules. The second option, emission of energy and subsequent reabsorption will occur for larger distances and it is critically dependent on resonance. The third option, *internal conversion*, occurs when the separation distance is large compared to the atomic dimensions, but small compared to the wavelength of light. Since the distance between the dye and the chlorophyll molecules is in the order of 5×10^{-7} cm, and the assumed efficiency of the energy transfer mechanism is more than 90 percent, the authors ruled out the possibility of mechanisms (1) and (2) and posited that *internal conversion* is the correct mechanism of energy transfer.

The authors then elucidated the mechanism of *internal conversion* and its high efficiency in nuclear physics. They stressed this critical fact: "energy transfer mediated

²¹ One mechanism for an excited state nucleus to make the transition to a state of lower energy is the emission of a γ -ray whose energy corresponds to the energy difference between the two states. Another competing de-excitation process is called *internal conversion*; an excited state nucleus decays to a lower energy state by the direct ejection of one of its electrons [Bertulani 2007]. In this case a nucleus in an excited state can transfer its energy via a nonradiative process to an electron in the K-shell with the subsequent ejection of the K-shell electron.

by time-varying electromagnetic fields will be enormously enhanced if the absorbing oscillator lies within a small distance [near field] as compared to the wavelength of the emitter. This is exactly the effect that accounts for the very large photoelectric absorption of nuclear gamma rays by tightly bound electrons in the surrounding atom”.

They further explained the analogy between *internal conversion* and resonance energy transfer in photosynthesis: “the electric field of an oscillator that is emitting primarily electric dipole radiation, which in the wave zone [far field] falls off linearly with the inverse distance from the emitter, increases, as we approach the emitter within distances small compared to a wavelength [near field], like the inverse cube of the distance”. These authors stressed the similarity between “*internal conversion* [nuclear physics] and the resonance transfer of energy from one oscillator to another oscillator in resonance with it and lying within the quasi-static [near field] rather than the wave zone [far field] of the former”.

They derived an equation in which the rate of energy transfer between the dye molecule and the chlorophyll molecule is a function of the distance of separation: R^{-6} . Finally, the authors stated that their proposed mechanism of resonance energy transfer is very similar to Förster's theory of resonance energy transfer and it predicted an efficiency of energy transfer that accounted for the photosynthesis efficiency that was observed previously in *Chroococcus* [Emerson and Lewis 1942].

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