THE HISTORY OF FRET:

From conception through the labors of birth

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1.1. INTRODUCTION

This chapter is an excursion into the historical development of energy transfer. This chapter is not concerned with a detailed review of applications, or a review of modern theoretical developments; this is available elsewhere (Van Der Meer *et al.*, 1994; Wu and Brand, 1994; Clegg, 1996). The topic is the emergence of Förster resonance energy transfer FRET. I also examine the ideas, experiments and theories that formed the scientific backdrop that preceded and led up to FRET.

FRET is a physical process whereby the excited state energy of one chromophore molecule, the "donor", can be transferred to a neighboring chromophore, the acceptor, in the ground state. This can take place whenever the two molecules are close enough, usually separated by less that 7 nm provided certain other conditions are met.

FRET is one of the major experimental methods for discovering whether two molecules are in close proximity, or for determining the distance between two specific locations on macromolecules and in molecular complexes. Energy transfer is used to follow conformational changes of macromolecules, either statically or in real time. It has recently become a major experimental technique in the field of single molecules. Since the "efficiency" of energy transfer (that is, the fraction of energy absorbed by the donor that is transferred to the acceptor) is usually measured with fluorescence tools, and fluorescence is sensitive, specific and widely available, FRET has become very popular. The chromophores (donors and acceptors) that are used for accomplishing this measurement are usually attached (often covalently) to other macromolecules, such as proteins, nucleic acids, and lipids. The energy transfer can be detected relatively easily and it is often used qualitatively to signify intimate interaction

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between two "labeled" biomolecules. Sometimes one or both of the participating chromophores occur naturally in biological macromolecules, such as tryptophan or chlorophyll. However, the number and variety of synthetic fluorescence probes available for labeling has expanded tremendously in the last several years. Several readable reviews of FRET for a general audience are readily available (Clegg, 1992; Van Der Meer et al., 1994; Clegg, 1996; Clegg, 2004a).

The FRET measurement is now applied routinely with a wide variety of samples: micro structures (such as DNA and protein chips and micro/nano assay arrays), living biological cells, and even whole organisms. It is a very powerful technique, fairly simple, and can be carried out in most laboratories with their existing spectrometers and microscopes. Although the technique has been readily available and applied since the early 1950s, the use of FRET has literally exploded in the last few years, in academic research as well as industrial applications, especially in biotechnology and bioengineering. This flurry of activity has many reasons. First, FRET measures interactions and dynamics on a spatial scale that is unique. Also, our ability to produce well defined and pure macromolecules in the laboratory has increased dramatically in the last few years, and it is relatively easy to label them specifically with fluorophores. In the last several years we have developed the ability to produce hybrids of specific proteins with fluorescent proteins (for instance, GFP, YFP, CFP and RFP, respectively green-, yellow-, cyan-, and red-fluorescence proteins) that can be produced in vivo under genetic control in the living cell (and in tissue); certain pairs of these proteins can undergo FRET. These fluorescence proteins have revolutionized the field of biological fluorescence, especially the measurement of FRET, in the fluorescence microscope. A great number of excellent synthetic fluorophores are available commercially, with the required chemical groups attached for specific labeling to biomolecules. In addition there have been many instrumentation improvements and innovations that make the FRET measurement much more sensitive and convenient. These chemical, biological, and instrumentation advances have expanded tremendously the range of applications, and the ease of carrying out the experiments.

In spite of the wide spread use of such a well known and useful technique, and the availability of several excellent treatise and reviews of the underlying theory, not to mention the hundreds of experimental applications published every year, little is published about the historical development of the major concepts. The historical events are not only interesting in themselves, but understanding and appreciating the major theoretical insights realized by the pioneers of energy transfer, and the scientific context in which they worked, provides insight into the mechanism, and leads to a better appreciation of the original contributions. A short history of the contributions of the Perrins and Foerster to FRET has been published recently (Clegg, 2004b). This chapter is a more extensive examination of the state of affairs and the general state of knowledge that was prevalent in physics at the time, leading up to the first observations and theoretical explanations of energy transfer.

1.2. PRELUDE TO THE HISTORICAL BACKGROUND

Although the practical applications of FRET started after 1950, the awareness that energy could be transferred between two atoms or molecules over distances larger than their physical collisional radii took place much earlier. The first experimental observation of energy transfer happened after 1900 and this chapter will only deal with FRET literature between 1900 and 1970. However, I will follow the thread back into the 19th century, when the ideas of an electromagnetic (EM) field and spectroscopy were being formed, and the dilemmas leading to quantum mechanics (QM) at the turn of the century were starting to appear. These theoretical concepts were essential for the observation and correct interpretation of non-radiative energy transfer. The notion of EM fields entered compellingly into the mainstream of physics only a few decades before the first observations of energy transfer. The first theoretical attempts explaining FRET were applications of this classical EM theory. And the first quantum mechanical theories of FRET were developed concurrently with the new theories of Heisenberg, Schrödinger and Dirac (Heisenberg, 1925; Dirac, 1926; Schrödinger, 1926b). So we will take a look at the historical scientific context in which the first experiments and theoretical accounts of energy transfer took place.

The aim of the first part of this chapter is to indicate the scientific atmosphere in which the idea of energy transfer at a distance was born. We start by taking a short journey through the development of the concept of electromagnetic fields, move quickly through the quantum theory of atoms and spectroscopy, and then into the first experimental and theoretical discoveries of energy transfer. This initial time frame is from 1820 to 1920. I have decidedly selected the topics, emphasizing only those concepts important for FRET. After reviewing the emergence of these physical concepts, this chapter will only deal with FRET literature between 1920 and 1960.

1.2.1. The End of the Dark Ages: the Pre-Field Era

The fundamental paradigms of physics were undergoing radical changes in the 19th century, and these ideas were critical for understanding that atomic (molecular) interactions could extend over distances long compared to their atomic (molecular) radii. A lively account of how the concept of a field in a void entered into physics is given in an easily readable account by Einstein and Infeld (Einstein and Infeld, 1966). The concept of electric and magnetic fields is of course now common place, but we will see it was not until Maxwell that this concept was set on firm footing.

The notion that magnetism and electricity were somehow related had been suspected for some time before 1800, because of the formal similarities between static electricity and magnetism. Hans Christian Oerstead[2] in 1820 was the first

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[2] Oerstead, a professor of Natural Philosophy in Copenhagen, received his PhD in 1799 in the medical faculty of Copenhagen; his topic dealt with Kant's philosophy.

to demonstrate the interrelationship of magnetism and electricity[3]. In a short four page article written in Latin (Greiner, 1986) he reported that a magnet's needle held next to a current-carrying wire was deflected, and oriented itself perpendicular to the line of current[4]. This interaction happened at a distance, decreased in effectiveness with increasing distance, and surprisingly the force on the needle was *perpendicular* to the line between the wire and the magnet[5]. This discovery, easy to reproduce[6], was the first direct demonstration of the connection between electricity (a current) and magnetism, and it was first done by accident at the end of a lecture demonstration (Whitaker, 1989a). He wanted to show that if the magnet was perpendicular to the current flow there was no effect (which was true). At the end of the lecture he inadvertently oriented the magnet parallel to the wire, and there was a pronounced deflection. It was a phenomenally significant and completely unanticipated discovery, especially since magnetism had been known from antiquity, and was conceived by many as somewhat magical with supernatural powers[7], and the compass and its use for navigation had been known for a long time. Oerstead's short report instigated immense interest throughout Europe, not only in the physics community, but was also enthusiastically received by workers in all scientific (and medical) disciplines. At that time, science was not as topically separated and divided as now. Even the general public heard of, and enthusiastically discussed, his experiment. Interestingly, Oerstead wrote later that it was his interest in romanticism and the movement of romantic natural philosophy that inspired him to carry out these experiments. He was also a passionate and tireless lecturer, and this may explain some of his influence (although, apparently, some scientists of that day did not appreciate his romantic outlook, and thought of him as a lucky, amateurish, dreamy opportunist – he earned handsomely from this discovery). Whatever, one might say that this was the inauguration of a great paradigm change in physics.

Two months later it was announced in Paris by Dominique François Jean Arago[8], the famous French astronomer, who had just returned from Denmark. André-Marie Ampère (and others, e.g. Jean-Baptiste Biot and Félix Savart)

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[3] Of course, this discovery could not take place before one had the ability of making currents (Voltaic piles) and had wires. Both these requirements had only been available since approximately 1800.

[4] Oerstead got the idea to carry out his experiment with a galvanic circuit because it was known that deflections in a compass needle take place during lightning bursts in thunderstorms.

[5] Oerstead did not determine quantitative aspects of this discovery; this was done by others soon after his discovery.

[6] Interestingly, Oerstead was apparently all "thumbs" in the lab, and all his experiments had to be carried out by his (enthusiastic) students and assistants.

[7] Magnets had been purported since antiquity to have healing powers. In 1780-1800 Franz Anton Mesmer, a doctor considered a charlatan by many but a medical savior by others, became a sensation "mesmerizing" his patients by passing magnets – in the appropriate mystifying setting – over the location in their body of their suspected ailments (or heads, if they were mentally distressed). To be fair, he realized later that his method had to do with suggestion, and did not require magnets. His work was the forerunner of the later work of Puysesur, Braid, Charot and Freud on hypnosis.

[8] Arago discovered in 1811 the rotation of the plane of polarization when polarized light passes through an optically active crystal.

immediately repeated Oerstead's experiments. Ampère was adept in the manipulation (and participated in the theoretical development) of partial differential equations (he was also well educated and took part in the world of literature and philosophy), and in 1822 he soon came up with a theory of electromagnetic interactions involving currents[9]. He found that current-carrying wires would attract or repel each other depending on whether the currents were in the same direction or opposite. As a result of his interest in Oerstead's experiments, he suggested the possibility of a telegraph together with Jacques Babinet. This is the *first mention that communication between two places could take place via electromagnetic interactions*[10]. I mention this, because electromagnetic communication between two locations is the basic physical event in FRET.

1.2.2. Middle Ages: Experiments That Eventually Changed Our World View

In the realm of magnetism and electricity, the pictorial, intuitive representation of lines or tubes of force was introduced by Faraday in 1821 (Whitaker, 1989a) [11]. Faraday was perhaps the most thorough, dedicated, likable and honest of all experimenters. His interest in the inter-conversion and transformation of forces between different forms was a lifelong goal (this was before the concept of the conservation of energy and the equivalence of heat and energy by Julius Robert Meyer in 1842). Faraday handled his lines of force as though they were real physical entities, and not just as abstract helpful mathematical concepts[12]. He pictured these lines of force as the mechanism by which electrical and magnetic substances interact with themselves and with each other; these tubes of force were so to speak for him the carriers of forces through space. He discovered the "Faraday induction" (i.e. changing magnetic fields produce circulating electrical fields), which is the basis of modern electric motors. The idea of forces at a distance had of course been a topic for a long time (e.g. Newton and gravitation). However, Faraday's ideas of tubes of force

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[9] Ampère's first law gives the force on a current carrying wire placed in a magnetic field; his second law is that the magnetic field $\vec{B}$ circulates around an enclosed electrical current $\vec{j}(\vec{r})$; that is, in vector notation, $\nabla \times \vec{B} = \left(4\pi/c\right)\vec{j}(\vec{r})$

[10] The electromagnetic telegraph was later implemented by Karl Friedrich Gauss and Wilhelm Edward Weber in 1833, who developed the practical telegraph; they sent the first telegraph message in Göttingen from Gauss's observatory outside the city limits to Weber's laboratory in the city. Interestingly, there was much debate among the population of Göttingen whether the wires strung over the house tops were possible health hazards because of the magnetic effects emanating from the current carrying wires. Gauss developed a system of units for measuring magnetism that was based on length, mass and time, and this is the basis for the unit system in EM called the Gaussian Units. The unit Gauss, measures the strength of a magnetic field.

[11] Actually, this concept was reported by Niccolo Cabeno, as early as 1628, and referred to as early as 1629 by Aristotelian-scholastic philosophers.

[12] Faraday had no knowledge of formal mathematics. It has been mentioned to me by a mathematical physicist that he was perhaps the supreme mathematical physicist, with an incredible spatial imagination that did not need the crutches of equations.

did not sit well with many of the mathematical physicists of the day, who were used to expressing all in terms of differential equations[13]. Nevertheless, he was an exceptionally capable experimenter; his experiments and graphical explanations were remarkably innovative and provided astounding, intuitive insight. His ideas set the stage for the next step important for the background for FRET, which was then carried out by Maxwell.

1.2.3. Renaissance: Enter the Theory of Electrodynamics and Fields

The basic FRET phenomenon involves the electrodynamic interaction between two molecules over distances that are large compared to their diameters; and this description requires the idea of an EM field (for FRET this is a dipole interaction, which arises from a multi-pole approximation to the Coulomb interaction). Faraday did not deal with fields, but with tubes of force. It was James Clerk Maxwell who introduced the first field theory. His equations describe the EM field; the objects (electrical or magnetic) enter only through boundary conditions. The ideas and experiments of Faraday played a major role in Maxwell's theoretical development. He created a complete mathematical representation of Faraday's descriptions of electricity and magnetism (Maxwell, 1873; Simpson, 1997). He admired the work of Faraday, and read all what Faraday had written before undertaking the task of formulating his ideas in mathematics[14]. He was of the opinion that Faraday had articulated his discoveries "in terms as unambiguous as those of pure mathematics". Maxwell's accomplishment is enshrined in his famous classical equations of electrodynamics, which are familiar to all physics students (and often the cause of much sweat and toil). In addition to the concepts he borrowed from Faraday, he introduced the notion of displacement current – the circulatory magnetic field caused by a time-varying electric field (also in empty space). These equations describe all classical electrodynamic phenomena, and they are the starting point for describing energy transfer. His equations furnished the theoretical setting to predict electromagnetic radiation (e.g. the classical theory of fluorescence emission).

It is a fascinating story how Maxwell wrestled with physical and mathematical analogies, experimental results and mathematical formulations in order to arrive at his equations, as well as how his equations predicted light as an electromagnetic field. He created the term electrodynamics, as this quote from him exemplifies: "The theory I propose may therefore be called a theory of the *Electromagnetic Field*, because it has to do with the space in the neighborhood of the electric or magnetic bodies, and it may be called a theory

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[13] Faraday eventually repudiated a reality that consisted of separate entities of matter (atoms) and void: "The difference between a supposed little hard particle and the powers around it, I cannot imagine".

[14] The titles of Maxwell's first two of his three main papers on EM were: "On Faraday's Lines of Force" (1855) and "On Physical Lines of Force" (1861). The first shows his respect and enthusiasm for Faraday's ideas, and the second signifies his new paradigm of the physical field concept. His third paper on this subject "A Dynamical Theory of the Electrodynamic Field" was presented in (1864).

of the *Dynamical Theory*, because it assumes that in that space there is matter in motion, by which the observed electrodynamic phenomena are produced" (this quote is from his third paper in this series – "A Dynamical Theory of the Electrodynamic Field"). This is actually a partial description of what happens in FRET. Maxwell understood Faraday's lines of force as "a line passing through any point of space so it represents the direction of the force exerted ...". This depicts nicely the vector representation of the EM field, which is now given in every EM textbook, and is the way the orientational dependence of the interaction between a FRET pair is portrayed.

The impact on the physics community and the conceptual revolutions that were initiated by Faraday and formulated by Maxwell are perhaps difficult to appreciate[15]. The theory of Maxwell, based on the original ideas of Faraday, turned much of physics on its head. Whereas Newton's laws conserve energy and momentum in the motions and collisions of bodies, Maxwell's field theory is concerned with the energy and momentum of the field, and does not take account of the bodies, except as boundaries. Because this concept of a field is so critical for understanding FRET, and this is a historical account, I emphasize this with two quotes. The first is from Ludwig Boltzmann[16], who, to express his admiration for Maxwell's equations, quoted Goethe: "War es ein Gott, der diese Zeichen schrieb?" – Was it a god, who wrote these expressions?". The second is from Maxwell himself[17]: " In speaking of the Energy of the field, however, I wish to be understood literally. All energy is the same as mechanical [...]. The energy in electromagnetric phenomena is mechanical energy. The only question is, Where does it reside? On the old theories it resides in the electrified bodies, conducting circuits, and magnets, in the form of an unknown quality called potential energy, or the power of producing certain effects at a distance. On our theory it resides in the electromagnetic field.". This was an enormous paradigm change. The difficulty to imagine energy in a void led to the introduction of the "ether". Ether was supposed to be the inert medium through which all electric and magnetic phenomena were transmitted. This was not required by Maxwell's equations. But scientists trained in the Cartesian view of physical phenomena found the ether necessary (including Maxwell). It was difficult to imagine how the transverse undulations of light (required and predicted by Maxwell's equations and verified by Hertz) could take place without a medium. As we know, the ether was shown not to exist. (Whitaker, 1989a; Whitaker, 1989b).

1.2.4. The Beginning of the Modern Age: The Field Surrounding an Oscillating Charge

The next critical step for FRET was carried out by Heinrich Hertz, with his famous Hertzian oscillating dipole. The electrodynamic field emanating from a vibrating electric dipole (the Hertzian oscillating dipole) is derived from

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[15] Although eventually this impact was prevailing, Maxwell's EM theory was not immediately accepted, and was even highly criticized my many scientists.
[16]" Vorlesungen über Maxwells Theorie", L. Boltzmann
[17] "A Dynamical Theory of the Electrodynamic Field" JC Maxwell (1864)

Maxwell's equations. This is the classical theoretical basis for understanding the production of light (e.g. emission of fluorescence from atoms and molecules, as well as the absorption), and is also the *starting point of the first classical descriptions of FRET*. Maxwell's equations predicted the identity of electromagnetism and light (expressed in his memoirs of 1868), and even foretold the quantitative properties of light (interference, refrangibility, polarization, as well as the speed of light). This was brilliantly confirmed by the experiments of Hertz. These experiments, and his theoretical description based on Maxwell's theory, forced the skeptics in the scientific community to accept the concepts inherent in Maxwell's field equations. Hertz carried out the critical experiments in 1888, and published the theory (derived from Maxwell's equations) to explain the EM fields surrounding his electric oscillator in 1889. This derivation is given in any intermediate or advanced electrodynamics textbook. Of course, Hertz was not specifically referring to an atomic oscillator, but to a macroscopic electric oscillator.

His first experiments were carried out by producing high frequency repetitive sparks in an air gap of a primary oscillating circuit (which acted as the source of the EM radiation). The electrodynamic disturbance was detected at a distance by a secondary circuit, *resonant* with the first, with a similar air gap. Sparks were observed in the secondary receiving circuit when it was resonant with the primary circuit. At first Hertz was primarily interested in proving the existence of propagating electromagnetic radiation (light, at frequencies of what is today radio frequencies), which was predicted by Maxwell's equations. Therefore, in the first experiments the distance between the primary and secondary circuit was long compared to the wavelength of the propagating Maxwell electromagnetic wave at that frequency of oscillation. The result was fully consistent with Maxwell's field equations, and this is of course the basis of all radio communications.

Hertz's theoretical description describes the electromagnetic disturbance in the near field (much less than a wavelength of the emitted radiation), in an intermediate zone, as well as in the far zone (at distances greater than a wavelength) where the electromagnetic energy escapes and is carried away as radiation with transverse oscillations. He calculated a very graphic field-line representation of the EM field of an oscillating dipole, demonstrating how the field lines are pinched off at approximately a distance of a wavelength, at which point transverse waves in the far field are formed (E and B fields of propagating radiation are perpendicular to the direction of photon travel). Only in the far field can we think of a photon (or in the language of pre-photon concepts, a traveling light wave). In the near field (distances small compared to the wavelength, which is where FRET takes place) both transverse and longitudinal components of the EM fields are present. However, in spite of the high energy density in the near-field, no propagating EM waves are present here (no emission of energy). This corresponds to the terminology in FRET that the energy in the near field is transferred non-radiatively. Experiments carried out in the near field verified Hertz's theoretical description.

The graphical and mathematical description of the oscillating electric field emanating from a Hertzian dipole, in particular in the near field, played a

critical role in the understanding, and the eventual theoretical description, of FRET. The oscillating E-field in the *near field* of a Hertzian dipole has the same effect on a receiving oscillator as when the receiver is in the *far field*. The only difference between the near- and far-field effects on a receiver (acceptor in FRET) is the direction of the field vector (and the intensity), which is a sum of tangential and longitudinal components in the near field, and only tangential in the far field. The oscillating electric field surrounding the Hertz oscillator is indispensable for all theoretical descriptions of FRET.

1.3. FIELDS, SPECTROSCOPY AND QUANTUM MECHANICS

1.3.1. Fields

As we have seen, in a relatively short period of time the paradigm in physics that all interactions take place by mechanical contact and collisions, changed considerably (Einstein and Infeld, 1966). By 1900 the concept of a field had been generally accepted (this is only 22 years before the first report of energy transfer at a large distance between atoms in a vapor (Cario, 1922)). Nevertheless, because of the very successful dynamical gas theory (the groundwork of which was also set by Maxwell), most original interpretations of energy transfer and fluorescence quenching between gaseous atoms naturally assumed collisional contact (mechanical interactions).

1.3.2. Quantum Mechanics and Spectroscopy

At the turn of the century there was another paradigm change in physics about to take place, which is the second requirement for understanding FRET. This took place because the theory of radiation, in spite of the success of Maxwell's theory, had reached a very unsatisfactory state. This had to do with the failure to explain the dispersion (frequency dependence of the energy emission) of blackbody radiation. As is well known, in 1900 Planck solved this problem by introducing the quantum concept – energy changes *in matter* could only take place in well defined quantum jumps. His successful theory for explaining black body radiation was presented December 14, 1900 at the German Physical Society (Planck, 1900) [18]. His reasoning centered on an ensemble of *Hertzian oscillators* (the oscillators were the atomic constituents of the walls), which he proposed could only exchange energy with the radiation *field* in jumps of energy quanta. His famous paper was published in 1901 (Planck, 1901). This was followed by the work of Einstein (Einstein, 1905;

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[18] In October of 1900 he had presented to the same society a phenomenological theory that was in agreement with black body experiments, which was a modification of previous work of his (in 1899) based on thermodynamic reasoning; but he considered this approach unsatisfactory because it contained undefined empirical constants.

Einstein, 1906), where he proposed that light itself behaved as though it were particle-like quanta of energy[19].

1.3.2.1. Conway and Ritz

Up until 1907, the picture that scientists had of absorbing and emitting atoms was that each atom consisted of an electrical system with many natural periods of oscillation, all present simultaneously. In 1907 Arthur William Conway, from Dublin (Conway, 1907), proposed that the spectrum of an atom does not result from free vibrations as a whole, but that each atom produces spectral lines *one at a time* (that is, the actual spectrum observed at any time depends on the presence of many atoms). This is prior to the Bohr-theory of the atom, or even of the Rutherford model of an atom (1911). The idea of Conway was that in order to produce a spectral line, one electron in an atom must be in some sort of perturbed state (he had no way to describe this in detail), and that this electron is then stimulated to produce vibrations of a frequency corresponding to the observed spectral line. This disturbed state did not last indefinitely but would decay with time, emitting a fairly long train of vibrations (as was required by Maxwell's equations). This was remarkable insight considering that he did not know the later interpretations of atomic spectra (e.g. the Balmer and Rydberg series) in 1908 by Ritz (Ritz combination principle) (Ritz, 1908) who showed how the spectral lines could be interpreted by *differences*, taken in pairs, of certain distinct numbers.

1.3.2.2. Bohr

This discussion would not be complete without mention of the critical insight of Niels Bohr (Bohr, 1913; Hettema, 1995), who integrated and selected many of the ideas that were being considered at the time (including those of Conway (Gillispie, 1960)). He selected what he considered to be superior ideas from the inferior ones, and produced the paradigm of the atom (the Bohr atom) that was enormously influential and placed spectroscopy on firm ground. This is now so common-place and is even sometimes introduced in grade school; however, his synthesis of a model that could explain quantitatively the spectroscopy of many simple one-electron systems was a real eye-opener, and was critical for all that followed. Because it is so well known, I spend no time describing Bohr's ideas. Of course, extensive experimental studies in spectroscopy were carried out at that time (Pringsheim, 1928). The spectroscopic experiments stimulated the theoretical work, and provided the data for motivating and checking the theories. The extensions of these basic ideas to complex molecules could only take place after the introduction of quantum mechanics by Heisenberg in 1925 (Heisenberg, 1925) and Schrödinger

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[19] Although Planck had considered that energy of the field could be quantized, he resisted this because this would go against all that was known of the "continuous" Maxwellian light field; for this reason he only considered the quantized emission of energy from the oscillators, and not the absorption.

in 1926 (Schrödinger, 1926b), and we will see in the next section that the new quantum mechanics was immediately applied by Kallmann and London (Kallmann and London, 1928) to explain energy transfer in vapors, and by F. Perrin for energy transfer in solution (Perrin, 1932).

1.4. THE FIRST EXPERIMENTAL OBSERVATION OF ENERGY TRANSFER AT A DISTANCE – SENSITIZED LUMINESCENCE IN VAPORS

1.4.1. Sensitized Fluorescence

With the concept of EM fields, the experiments and theory of Hertz, the development of the older quantum theory, the spectroscopy data of atoms, and Bohr's theory of the atom, we have reached the point of entry for FRET. The first recorded measurements of energy transfer (observing the emission of the accepting atom) over distances larger than collision radii were made in 1922 by Cario and Franck (Cario, 1922; Cario and Franck, 1922; Franck, 1922). Cario observed emission from thallium in a mixture of mercury vapor and thallium vapor, when the vapor mixture was excited with wavelength of 253.6 nm, which can only excite the mercury atoms. This fluorescence emission from thallium was named "sensitized fluorescence". It was obviously due to the transfer of energy from the excited mercury atoms to the thallium atoms. Further experiments by many people showed sensitized fluorescence with the vapors of the alkali metals: silver, cadmium, lead, zinc and indium in the presence of mercury vapor. The importance of resonance between the energy levels of the sensitizer and the sensitized atoms was explicitly shown by further experiments, especially with the later experiments of Beutler and Josephi (Beutler and Josephi, 1927; Beutler and Josephi, 1929), who studied the sensitized fluorescence of sodium vapor in the presence of mercury vapor. The sensitized fluorescence increased in intensity the smaller the energy differences between the states of the two participating atoms. This was consistent with "Franck's principle" (Franck, 1922), which had been articulated, in reference to fluorescence quenching, previous to the experiments of Cario. This principle can be stated as: "the electronic energy of an excited atom cannot be transferred directly into kinetic energy of the colliding particles. If the excitation energy has to be taken over almost completely as internal energy of the quenching molecules, these must have some sort of excited states, which *are in energy resonance* with the primarily excited states", page 116 of Pringsheim's book (Pringsheim, 1949); my italics. Here we see already the *entrance of "resonance" into the FRET story*, which will play a central role.

Sensitized fluorescence was discovered during the copious spectroscopic experiments that were carried out at different temperatures and densities with vapors of many different atoms and diatomic molecules in the first two decades of the twentieth century. A thorough discussion of literature up to 1949 can be found in Pringsheim's treatise (Pringsheim, 1949). Dynamic fluorescence

quenching (due to collisions), photochemical reactions, resonance fluorescence and molecular associations were intensively studied in the early decades of the 1900s (Wood, 1934). Many of these experiments were naturally interpreted in terms of collision theory. The number of collisions per time could be calculated simply from gas theory; and the fraction of collisions that were effective (leading to quenching, chemical reactions, or sensitized fluorescence) could then be determined.

1.4.2. Spectroscopic and Collisional Cross-Sections in Vapors

The cross-section of molecular encounters (or what is equivalent, the frequency of effective collisions) gives estimates for the "spectroscopic size" of the reacting atoms. From the fraction of successful collisions, calculated by comparing the rate of successful quenching encounters to the collision rate from gas theory, one can calculate a "*spectroscopic cross-section*". If the radius of this cross-section is smaller than the radius of the atoms or molecules (or equivalently, if the rate of collisions is smaller than calculated from the gas theory) then the conclusion is that only a certain percentage of the collisions are effective in quenching. If the spectroscopic cross section is larger than expected from the encounter radius, then it is assumed that there are interactions between the two collision partners that extend beyond their encounter radius. A larger radius of interaction than predicted from the theory of gas dynamics was found for many energy transfer measurements in the vapor. These large "spectroscopic" cross-sections constituted the first hint that many inter-atomic interactions could take place over larger distances. This discovery that energy transfer could take place over distances large compared to the encounter radii showed that hard physical collisions were not required for atoms (or molecules) to interchange energy.

1.5. THE FIRST QUANTUM MECHANICAL THEORY OF ENERGY TRANSFER

A quantum mechanical theory to explain the transfer of energy between atoms at longer distances compared to collisional radii was proposed by Kallmann and London[20] (Kallmann and London, 1928). This theory assumed "almost resonance" between the energy levels of the interacting atoms. Essentially this is a second order perturbation calculation to calculate the energy of interaction. I will use their notation in this paragraph. They found that provided the corresponding transitions between the energy states of the two atoms were (spectroscopically) dipole-allowed, the effective cross-section q of

the two interacting atoms increases as $\sigma^{-2/3}$, where σ is the difference between

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[20]This is the same F. London who proposed the quantum mechanical description of van der Waals interactions, which also involved dipole-dipole interactions, similar to FRET.

the excitation energies of the two interacting systems. As $\sigma \to 0$, the cross section approaches a limiting value much larger than the collisional radii. This work was the germ of the later quantum mechanical FRET theories, and is very similar to the description of London-van der Waals forces. Before discussing this paper further, we look at classical theories that were published just previous to Kallmann and London.

1.5.1. A Few of the Pre-Quantum Theories that calculated the Spectroscopic Cross-Sections of Atomic Vapors

Some of the previous pre-quantum papers (Holtsmark, 1925; Mensing, 1925; Nordheim, 1926) (all referenced by Kallmann and London) dealing with "spectroscopic cross-sections" are remarkable in their insight.

Mensing (Mensing, 1925) considered how intermolecular dipole-dipole interactions broaden spectra (using the Bohr-Sommerfeld atomic theory with elliptical orbitals) of atomic vapors. She derived a broadening due to dipole-dipole interactions to be approximately a width of $\delta v \approx 3.6 \left(e^2 / h \right) \left(a^2 / d^3 \right) \left(1 + 3\varepsilon^2 / 2 \right)$, where a and ε are the long axis and eccentricity of the atomic orbitals, e is the electron charge, and d is the distance between the two molecules. She considered dipole-dipole interactions of circulating electrons (from a semi-classical point of view, this is similar to what is done by London when the two interacting molecules are not in the same energy state).

An article by Nordheim (Nordheim, 1926) presents a collision theory between atoms, whereby the atoms interact via dipole-dipole terms. Although the new quantum theory of Heisenberg (Heisenberg, 1925) and Born and Jordon (Born and Jordan, 1925) had been published already, Nordheim calculates the interactions classically. He justifies this choice because the results should be approximately the same, and he also wanted to by-pass the difficulties in analyzing the collisions quantum mechanically. Later, Förster, Ketskeméty and Kuhn (Förster, 1951; Ketskeméty, 1962; Kuhn, 1970) showed that the classical and quantum calculations arrive at the same result. Nordheim derives also higher multipole interaction energies, and uses the same classical conjugate dynamic equations of motion as Mensing. By considering collisions between the electrical multipoles that are oscillating at spectroscopic frequencies, he derives expressions for the energy transfer rates proportional to the product of the appropriate powers of the spectroscopic transition moments of the different multi-pole interactions. However, this derivation is very complex, because the rate depends on very complex averaging of the collision paths, and the velocities. The details of the theory are only applicable to vapor samples.

Holtsmark's paper (Holtsmark, 1925) is a purely classical approach aimed at understanding the average deviation of the energy levels from that of the free atom values of Na-vapors, in order to understand the broadening of the spectroscopic lines. The spectral widths were known to be broader than calculated from simple hard-core collision theory according to gas theory. A

classical perturbation approach (assuming that the atoms are classical electron oscillators) was taken whereby again the interaction of the atoms was assumed to be dipole-dipole. Holtsmark calculated an interaction proportional to $1/R^6$ for the interaction between any two atoms. Since he was interested in a random collection of atoms at a certain concentration, he integrated all interactions from the shortest distance of atomic approach (d) to infinity (including the orientation factor). This results in broadening of the lines proportional to $\left(e^2/m_e\right)^2 / d^3$ (where e is the electron charge and m_e is the mass of the electron); although he has assumed perfect electric oscillators, each one contributing a factor of e^2/m_e, the interaction would also be proportional to the multiplication of the oscillator strengths. He did not assume exact resonance – the total interactions were summed via a type of overlap integral. His factor d^{-3} is the same dependence on the distance of closest approach as by Arnold and Oppenheimer (Arnold and Oppenheimer, 1950) (see below) for the rate of energy transfer in a condensed system with random acceptors.

The point of discussing these early theoretical accounts of dipole-dipole interactions leading to energy transfer is to show the type of analyses invoked at this time to account for the very large effective molecular spectroscopic cross sections. These cross sections were much larger than expected from simple atomic collisional gas theory. This could only be explained by molecular interactions at large distances. These theories were published just a few years before Kallmann and London's quantum derivation, and at the same time as J. Perrin's work on energy transfer in solution. Already at this time, it was apparent to everyone that dipole-dipole (or higher multi-pole) interactions could extend the radius of inter-atomic interactions considerably. Some of the theories showed explicitly the $1/R^6$ dependence. In addition, it was shown that the spectroscopic oscillator strengths (that is the spectra) had to overlap and the orientation of the dipoles were taken into account. It is clear that the pieces are starting to fall into place.

1.5.2. Some Details of the Kallmann and London Paper

The theory of Kallmann and London (Kallmann and London, 1928) dealt with energy transfer in vapors of atoms; their theoretical ideas were the basis of the later quantum mechanical theory proposed by F. Perrin (Perrin, 1932; Perrin, 1933) for energy transfer in condensed systems, which was subsequently improved and extended by Förster (Förster, 1948).

The article by Kallmann and London (K&L) has many interesting aspects that are important for understanding the later theoretical treatments of FRET. Because F. Perrin essentially used a very similar theoretical approach, and his theory is outlined in the section "F. Perrin's model" below, I refer the reader to that section for a discussion of the basic ideas of the derivation. K&L assumed that the two interacting atoms have two states, but the energy levels do not have

to be exactly the same. They write the differential equations derived from the Schrödinger wave mechanics pertaining to the coupled system for the case of two states (Schrödinger, 1927). The basic equations were given by originally by Schrödinger (Schrödinger, 1927), in an article titled "Energieaustausch nach der Wellenmechanik", or "Energy exchange according to wave mechanics". K&L refer to this paper (this will be important when we discuss Rabi oscillations below), and it is remarkable that Schrödinger essentially indicated the path for a solution to energy exchange between two atoms (molecules) in one of his first quantum mechanic papers. The solution for this problem, if the energy states are narrow, is oscillatory, and K&L give the probability that the system of two atoms will have transferred energy to the other, $\left|c^2\right|$, as (using their notation)

$$\left|c^2\right| = \frac{\beta^2}{1+\beta^2}\sin^2\left[\frac{\pi}{h}\sigma^*\sqrt{1+\beta^2}\,t\right] \qquad .$$

$\beta = 2W_{12}/\sigma^*$. W_{12} is the interchange integral of the perturbation W between the two states of the system. $\sigma^* = \sigma + W_{11} - W_{22}$. σ is the difference of the energy levels of the two participating molecules. They assumed for simplicity that the zero energy levels of both molecules were equal. W_{11} and W_{22} are the configuration integrals of the perturbation W for the two different states (the first order energy change from the perturbation). W is the dipole perturbation operator, and $W = \left(\mu_1\mu_2\right)/R^3$ where μ_1 and μ_2 are the transition dipole moments of the two molecules; this is the usual Coulomb interaction of dipoles (the QM version of the Hertzian dipole interaction energy). They are interested in collisions between atom gas molecules, and if the time for an atom to pass by another is long, compared to the oscillation of $\sin^2\left[\cdots\right]$ term, they can take an average over this time, giving them the probability ϖ that, on the average, the energy will be transferred from the first combined molecular state to the other state. This is (skipping several steps – and carrying through several substitutions in order to show the relationship between their derivation and Förster's later theory)

$$\varpi = \frac{1}{2}\frac{\beta_m^2}{1+\beta_m^2} = \frac{1/2}{1+\beta_m^{-2}} = \frac{1/2}{1+\left(2W_{12}/\sigma\right)^{-2}}$$

$$\varpi = \frac{1/2}{1+\left(2\frac{\mu_1\mu_2/R^3}{\sigma}\right)^{-2}} = \frac{1/2}{1+\left(\frac{R^6}{4\left(\mu_1\mu_2\right)^2/\sigma^2}\right)} = \frac{1/2}{1+\left(\frac{R^6}{R_0^{'6}}\right)}$$

I have defined the variable $R'_0 = \left(4\left(\mu_1\mu_2\right)^2 / \sigma^2\right)^{1/6}$ in anticipation of the normal R_0 of energy transfer. *Except for the factor of $1/2$, this is the standard expression for the efficiency of FRET.* The $1/2$ arises because the solution is oscillatory, and K&L have calculated the time average. In addition K&L have not integrated over a frequency spectrum of the two spectroscopic transitions, which in reality cannot be sharp lines (this would give us the overlap integral – see our discussion of Arnold and Oppenheimer's derivation (Arnold and Oppenheimer, 1950)). This equation by K&L is quite remarkable – it is the first indication, except for the classical calculations given in the last section, that for two atoms separated by R the probability of energy transfer obeys a $1/\left(1 + R^6 / R'^6_0\right)$ rule. The validity of the equation depends on the ratio of the energy differences and the size of the perturbation, and I cannot delve into the reasoning concerning this ratio when calculating molecular interactions in this paper (but see the short discussion by Knox (Knox, 1996)). The important point is that already in 1928 K&L derived essentially the correct dependence on the separation of the two molecules. By integrating this equation over R they arrived at their expression for the "spectroscopic" cross section q.

$$q = \int_0^\infty \frac{2\pi}{2} \frac{\beta_m^2}{1+\beta_m^2} R\,dR = 0.96\pi \left(\frac{\mu_1\mu_2}{\sigma}\right)^{2/3}$$

This is the effective cross-section of atomic collisions, which is significantly larger than the hard core cross sections due to dipole-dipole interactions between one excited molecule and one ground state molecule). The last equality holds for not too sharp resonance. This is the relationship $q \propto \sigma^{-2/3}$ given two sections previous. And it is based on an efficiency of energy transfer that varies as R^{-6}.

K&L calculate approximations to this integral, and find that the dipole-dipole interaction at a distance results in an anomalously large cross section, and their equation compares well to the experimental results. The above derivation is valid if the two molecules are not exactly in resonance (that is, if the spectroscopic transitions of the two molecules are not identical). K&L derive the case for exact resonance, and decide that this would give values R'_0 values far too large. This is partly because one would have to account for the unavoidable frequency spread of the molecular transitions. I discuss this below. This is essentially the same as the F. Perrin derivation below, and so I refer the reader to this section on F. Perrin for a discussion of this. We will see that even though F. Perrin used the same basic quantum calculation as K&L, which is that discussed by Schrödinger in 1927 (Schrödinger, 1927), he used exact resonance and calculated the rate of energy transfer in another way. F. Perrin calculated the rate from the period of the oscillation in the $\sin^2\left[\cdots\right]$ term of the above

solution of K&L; and this gives the wrong distance dependence of energy transfer. In summary, K&L had the right idea, as well as the right dependence on R.

1.6. LONDON FORCES (VAN DER WAALS) AND DEBYE AND KEESOM INTERACTIONS

Before delving further into theories of FRET, we turn our attention to a closely related topic – the quantum description of van der Waals (1873) interactions (or London dispersion forces). The idea of dipole-dipole interactions at a distance between atoms and molecules was being applied by London to explain intermolecular van der Waals interactions concurrently with his work on energy transfer (previous section). The calculation of classical descriptions of dipole-dipole and dipole-induced-dipole interactions had already been introduced to explain intermolecular interactions (Keesom, 1912; Debye, 1920; Debye, 1921).

There is a close connection and concurrent historical development of the theories describing London's intermolecular interactions and FRET. The major difference is that one is interested in the *energy of interaction* for the van der Waals forces, and in the *rate* of energy exchange for FRET. For normal van der Waals interactions, both interacting atoms are in the ground state[21]. In FRET, one of the interacting atoms (molecules) is in an electronically excited state. The theory of London's forces is also important with regard to the first quantum theories of FRET by F. Perrin.

1.6.1. London Interactions: Induced-Dipole-Induced-Dipole

Fritz London published his quantum mechanical description of these forces in 1930 (London, 1930; London, 1937). This was two years *after* he published the paper analyzing the transfer of energy between mercury and thallium with Kallmann (Kallmann and London, 1928). London's interaction energy is carried out by quantum mechanical second order perturbation theory. Normal London dispersion interactions involve fluctuating dipole-dipole interactions between atoms in their ground states.

An excellent review of classical and quantum mechanical theories of van der Waals interactions can be found in a 1939 review of Margenau (Margenau, 1939), and good accounts are also given in books by Davydov (Davydov, 1965) and Walter Kauzmann (Kauzmann, 1957). We limit our discussion to interactions between two atoms in their ground states; although, London forces

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[21] London forces usually refer to interactions between two atoms in their ground states; however, in the review by Margenau, he discusses the interaction between one atom in the excited state, and one atom in the ground state. This is essentially the same physical circumstance as FRET, except that London forces result from averaging rapidly fluctuating forces over time (and space), and one is looking at the energy of interaction, not a rate of transfer, which is what one is observing in FRET.

are summed to explain interactions between large, closely spaced macroscopic objects.

The expression for the London dispersion forces between two atoms involves the product of the corresponding visible and UV oscillator strengths of the optical transition for each interacting atom, just as FRET (Förster, 1951) (see section on Förster below). Usually the London dispersion energies are expressed in terms of the polarizabilities of the two atoms; however, the polarizabilities are related to the spectroscopic oscillator strengths (Margenau, 1939; Kauzmann, 1957). The separate components of the oscillator strengths are proportional to the squares of the (spectroscopic) transition moments for each optical transition of the atoms. The full expression for the London interaction is a sum over all significantly contributing optical transitions of the two interacting atoms (see the next paragraph, and see equation 7 of Margenau (Margenau, 1939)). If both atoms are in their ground states, the London forces are always attractive[22]. In addition, if the interacting atoms are not spherically symmetrical, and have anisotropic polarizabilities, their relative orientation will affect the interaction (just as the orientation dependence of FRET). The simple van der Waals interaction (not taking into account retarded potentials – which are involved in the Casmir effect (Casimir, 1948)) according to London's theory decreases as R^{-6}, where R is the distance between the atoms; this is the same distance dependence as FRET between two chromophores.

The interaction term in the total Hamiltonian is that of two interacting electrical dipoles, and is proportional to $1/R^3$. The London interaction energy is calculated using second order perturbation theory (the first order perturbation term for atoms or molecules in their ground states is zero). According to second order perturbation theory, the total energy of interaction is proportional to a sum over all higher energy states of the combined two atom system, where each term of the sum is proportional to the square of the interaction matrix element. Therefore, the total energy of interaction is proportional to $1/R^6$, where R is the distance between the two atoms. Each component of the sum is also proportional to the product of the squares of the transition dipole moments (these are identical to the spectroscopic transition moments) for each atom between the ground state and the excited state for that term of the sum. In addition, each term of the sum is divided by the difference between the combined energies of the two ground states and combined excited states corresponding to that term of the sum. Although the sum is extended over all higher energy states, the higher energy states are usually assumed to be small,

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[22] However, interestingly (especially within the historical context of FRET) if one interacting partner is in the excited state, the interaction can become repulsive. If one atom is in the excited state, the atomic interaction shows resonance; that is, the interaction becomes very strong when both the electronic transitions (absorption and emission oscillator strengths) have large values at very nearly the same frequency (energy). In addition, if one of the molecules is in the excited state, the interaction energy can vary as $1/R^3$, because the energy contribution from the first order perturbation calculation is no longer guaranteed to be small. This will become important when we deal with F. Perrin's FRET theory.

and are not included in calculations. This sum can be written in terms of the product of polarizabilities of both atoms. The denominator of each term is an energy difference, where the higher energy is subtracted from the lower energy. Therefore, each term contributes a negative energy component, meaning that the normal van der Waals interaction is universally attractive.

Although in many respects the theories of London interactions and FRET are similar, the two theories are not identical. One major difference is that the London interactions are calculated from second order time independent perturbation theory; this is the origin of the $1/R^6$ dependence and the squares of the transition matrix terms. The $1/R^6$ dependence and square of the transition matrix terms in the Förster expression for energy transfer emerge from different reasoning (application of Fermi's Golden Rule to calculate a rate), as I will discuss later. Fermi's Golden Rule can only be applied when the interacting oscillators are dynamically incoherent; this point will be important when we discuss the Perrins' treatments of energy transfer, because they did not find a $1/R^6$ dependence, but a $1/R^3$ dependence, essentially assuming a coherent interaction. However, as already mentioned, London found the correct $1/R^6$ dependence.

Since the London interactions and energy transfer interactions originate from dipole-dipole interactions, it is not surprising that Kallmann and London was working concurrently on both interactions. He derived both quantum mechanical theories for the vapor state essentially simultaneously. It is remarkable that he achieved this straight away so soon after the quantum theories by Heisenberg and Schrödinger.

1.6.2. Keesom and Debye Interactions: Dipole-Dipole and Dipole-Induced-Dipole

Previous to London's theory, inter-molecular interactions between molecular dipoles had already been proposed as the basis of molecular interactions. The Keesom orientation effect considered the interaction between *two* permanent *molecular dipoles* (Keesom, 1912); if the dipoles are strong, this can orient the interacting dipoles. Debye described induction forces between a *permanent molecular dipole*, and an *induced molecular dipole* (Debye, 1920; Debye, 1921); this is known as the Debye interaction or the induction interaction. For both the Debye and Keesom interations the energy varies as the inverse 6th power of the interatomic interactions, as do the London forces. The latter theory of London describes the van der Waals *dispersion* interactions as due to *fluctuating oscillating induced molecular electric dipoles* (London, 1930; London, 1937); that is, no permanent dipole need exist. All three interactions, dipole-dipole, dipole-induced dipole, and dipole induced – dipole induced, are usually included as components of van der Waals forces (Israelachvili, 1992). In contrast to the Keesom and Debye effects, the van der Waals interaction as described by London's dispersion forces is a pure quantum mechanical effect,

and is always present (although classical non-rigorous derivations are often given). The designation – *dispersion* - refers to the dispersion of light in the visible and UV spectral region (remember that the energy of van der Waals interactions can be expressed in terms of the oscillator strengths of the interacting molecules). As we will see, Förster's expression for the rate of FRET also involves the optical oscillator strengths (usually expressed in terms of the absorption and emission spectra).

1.7. FRET BETWEEN ORGANIC CHROMOPHORES IN CONDENSED SYSTEMS

We've now arrived at the time when the first attempts were made to explain observations indicating energy transfer over long distances in condensed systems (in solution). The first classical description of this by J. Perrin (Perrin, 1925; Perrin, 1927) predated London's quantum mechanical derivation of energy transfer in the vapor phase, but followed Cario and Franck's studies of energy transfer in vapors. As indicated above, dipole-dipole interactions were well understood at this time, and the description of the Hertz oscillator contains all the essentials needed to explain FRET on the basis of classical models of atomic electric oscillators. The classical description of FRET in condensed matter systems, with imposed restrictions from the old quantum theory, involves the comparison of the energy escaping to the far field from a Hertzian oscillator when it is alone, to the energy escaping when another molecule or atom (the acceptor) is in the near field zone. All the concepts that have been introduced above come into play: the oscillating electric field of a Hertzian dipole (near and far field), the quantum states of the oscillators (atoms), the requirement that the two communicating oscillators be in resonance and that the orientation of the two oscillators be favorable, and the idea of competition between emission of radiation and energy transfer. All these concepts were standing by, ready to be put into place when J. Perrin approached this problem for fluorophores in condensed solvents. However, because the new quantum mechanics had just begun, some rather subtle concepts in time-dependent quantum systems were not obvious.

1.7.1. Experimental Observations of Energy Transfer in Solution

The experiments that led J. Perrin to attempt a theoretical interpretation of energy transfer between molecules, involved fluorescence polarization in a solution of a single chemical species of fluorophore. It had been discovered by Weigert (Weigert, 1920) and by Gaviola and Pringsheim (Gaviola and Pringsheim, 1924) that the polarization of fluorescence emission from solutions of dye molecules began to decrease rapidly when the concentration was raised to a critical value (approximately 3 mM). This happened even when the fluorescence intensity (corrected for trivial absorption of fluorescence) was still linearly   increasing   with   concentration.   A   fluorescence   polarization

measurement determines the degree of rotational freedom of the fluorophores. If the fluorescent molecules are in a rigid environment or highly viscous solution so that they cannot rotate within the time of fluorescence decay (usually between 1-10 nanoseconds), the polarization contribution from individual molecules will have a high value (a maximum of 0.5). If the fluorescent molecules act independently, the polarization should be concentration independent. It could be shown for several well known dyes that the polarization was appreciably reduced when the molecules were on the average separated by about 50-80 Angstroms, much larger than the combined radii of the molecules. This distance was also much greater than the distance over which the excited fluorophores could diffuse within their excited state lifetimes (especially in high viscosity solvents, or solid solutions). This was the conundrum that led eventually to the discovery of FRET in condensed systems.

1.7.2. The Theories of J. Perrin and F. Perrin

1.7.2.1. J. Perrin's Model

A simple classical model to explain this polarization decrease was developed by J. Perrin (for details see below) (Perrin, 1925; Perrin, 1927). He hypothesized that the transfer of the excitation energy could hop from one molecule to the other through interactions between oscillating dipoles of closely spaced molecules. According to the classical theory of electrons in a molecule and the early quantum ideas, an excited molecule will oscillate at the frequency v corresponding to the magnitude of the excitation energy (the correct view according to the Bohr model is that the energy of the emitted light, $\Delta E_{exit} = h v$, is the difference between two energy levels, but it was known that the classical electron oscillator explained much of spectroscopy). Thus, he modeled the participating molecules classically as Hertzian electric dipoles (Hertz, 1888; von Hippel, 1954). As we know, close to a Hertzian dipole the oscillating electric field resembles a static dipole. Perrin assumed that if the molecules were separated by a sufficiently small distance, the energy could be transferred to the acceptor molecule non-radiatively. He called this "transfert d'activation". This is of course the same type of transfer considered by Mensing, Holtsmark and Nordheim (Holtsmark, 1925; Mensing, 1925; Nordheim, 1926) in their theories on atomic vapors. However, his derivation took quite a different route.

According to his model of two interacting *identical* Hertzian dipoles, Perrin calculated that this distance is approximately $\lambda/2\pi$, where λ is the wavelength of a free electric field oscillating at the frequency of the atomic electric field, v . $\lambda = c/v$ where c is the speed of light. A quantitative account is given below. Because he assumed that the two molecules were identical, λ is also the wavelength of the light used to excite the original donor. In J. Perrin's model the molecules had the *exact same frequency* of their electron oscillations; that is, the two Hertzian oscillators are in *exact resonance*. The two dipoles will

exchange energy similar to the resonance exchange of energy between two identical weakly interacting classical mechanical harmonic oscillators (just as two identical balls hanging on identical springs attached through a rod). Using the Hertzian dipoles as a model, and assuming *exact* resonance, he reasoned that if the molecules were separated by less than a critical distance (which he calculated to be $\lambda/2\pi$), one could detect this energy transfer between identical molecules by measuring a decrease in the polarization of the fluorescence emission, as had been determined experimentally. Because the acceptor would on the average not have parallel transition dipole as the donor, the fluorescence emission of the acceptor would be depolarized compared to the originally excited donors, and this would lead to a decrease in the measured extent of polarization. Already, we can see one problem; molecules cannot have exact resonance with each other at all times due to the uncertainty principle. In addition, their energies will also be broadened by collisions and thermal motion, and strong interactions with the solvent broaden the spectra considerably.

1.7.2.2. Where did the J. Perrin's Idea of Dipole-Dipole Interaction Come From?

As discussed above, the concept of interacting dipoles had been considered in other contexts for some time; so it was natural for J. Perrin to consider this once it was clear that the interaction between chromophores took place at a distance large compared to the molecular diameters. The classical model of absorption and emission of radiation, which involves oscillating dipoles (Kauzmann, 1957; Stepanov and Gribkovskii, 1968; Heitler, 1984), was also well developed by this time. Perrin's model is an application of these ideas of dipole-dipole interaction to the case where one of the molecules is in an excited electronic state, and the other is in the ground electronic state. He reasoned correctly that this dipole-dipole near-field Coulombic interaction could lead to the transfer of the excitation energy from the donor to the acceptor, without direct mechanical interaction of the two molecules and without the emission of a photon from the donor. Unfortunately J. Perrin's model says that the transfer can take place over distances of about 1000 Angstroms, which he realized was greater by a factor of 20 than the experimental results (see below). A subtle point here is that J. Perrin proposed that the intervening solvent (e.g. water at 55 molar concentration) did not participate in the "*transfert d'activation*". This is not a trivial point when we remember that the surrounding solvent did not play a role in the energy transfer experiments or theories in the vapor phase. In other words, he assumed that the solvent acted only as a dielectric bath.

1.7.2.3. F. Perrin's Model

F. Perrin (J. Perrin's son) was one of the pioneers of fluorescence (Perrin, 1929). He contributed extensively to the basic concepts. He extended J. Perrin's theory of energy transfer by developing a quantum mechanical model (Perrin,

1932; Perrin, 1933) (for details see below), similar to what had been suggested for transfer of energy between different atoms in gases (Kallmann and London, 1928). However, he concluded, as had J. Perrin, that the rate of transfer takes place proportional to $1/R^3$; this results in energy transfer at much longer distances than found experimentally. F. Perrin also later considered collisions between the chromophores and the solvent molecules, as well as Doppler effects (Perrin, 1932; Perrin, 1933). These collisions broaden the spectrum of the absorption and emission of the molecules, which had been originally assumed by J. Perrin to be infinitely sharp in order to guarantee effective interaction. Such collisional and Doppler effects had been the subject of much spectroscopic research on vapors in the first two decades of the 19th century, and had also been considered by Kallmann and London in the vapor phase. The broadening of the spectra is important for the following reason (this will also play a central role in Förster's theory). The energy lost by the donor must exactly equal the energy gained by the acceptor. The probability that the energy levels of the donor and acceptor molecules will simultaneously have exactly the precise values necessary to conserve energy during the transfer is much less than one. Each molecule has only a certain probability (weighted by the spectral dispersion) of being anywhere within the small spectral distributions caused by the collisions. This decreases the probability of resonance, because the two interacting dipoles must be closer than found by J. Perrin for a successful transfer of energy to take place. F. Perrin used the known theory of spectral collisional broadening to show that in the case of collisions the new distance for energy transfer is reduced to approximately $\left[\left(\lambda/2\pi \right)\left(\overline{t}/\tau \right)^{1/6} \right]$, where $\overline{t}$ is the time between collisions of the solvent with the molecule (at most $\approx 10^{-13}$ sec), and τ is the fluorescence lifetime ($\approx 10^{-8}$ sec). This would reduce the distance to about 200-250 Angstroms, which was still much too long, and would lead to transfer at about 100 micromolar concentrations, instead of the experimentally determined 3-5 millimolar. In addition, the theories of the Perrins (classical and quantum mechanical) did not provide a simple means to interpret the solution experiments. This discrepancy remained a puzzle for about 20-25 years, perhaps so long due to the Second World War.

1.7.3. A Derivation of the Perrins' Estimated Distances for Two Electron Oscillators in Exact Resonance

I have given in the previous sections the results of the Perrins' calculations; the following two sections are for those more quantitative aficionados who are curious how one can arrive at their answers. I only outline the basic line of attack. The reader who is not interested in these quantitative calculations can skip the next two sub-sections; but an understanding of the dynamic rates of energy transfer when the two molecules are limited to two states, and when the molecules are in exact resonance, is central for understanding why the Perrins

calculated such long distances. This will also be important when I present the major contributions of Förster.

1.7.3.1. The Classical Derivation of J. Perrin for two Hertzian Oscillators in Exact Resonance - with a Pinch of Old Quantum Theory

The electric field $\vec{E}_D$ in the *near field* zone surrounding a "donor" oscillating dipole has the same form as the field of a static dipole. This is

$$\vec{E}_D = \frac{1}{n^2 R^3} \left[3 \left(\vec{\mu}_D \cdot \hat{R} \right) \hat{R} - \vec{\mu}_D \right]$$

Where n is the index of refraction, R is the vector from the dipole (assume to be a point dipole) to the point of observation, and $\vec{\mu}_D$ is the dipole moment. The arrows indicate a vector quantity, and the carrot signifies a unit vector. If another dipole (the acceptor μ_A) is placed in the $\vec{E}_D$-field, the energy of interaction E_{ia} is

$$E_{ia} = -\vec{\mu}_A \cdot \vec{E}_D = \frac{1}{n^2 R^3} \left[\vec{\mu}_A \cdot \vec{\mu}_D - 3 \left(\vec{\mu}_D \cdot \hat{R} \right) \left(\vec{\mu}_A \cdot \hat{R} \right) \right] = \kappa \mu^2 / n^2 R^3 \quad ,$$

where the last equality is because in J. Perrin's theory the donor and acceptor dipoles are assumed to be identical. κ is the orientation factor,

$$\kappa = \left[\hat{\mu}_A \cdot \hat{\mu}_D - 3 \left(\hat{\mu}_A \cdot \hat{R} \right) \left(\hat{\mu}_D \cdot \hat{R} \right) \right].$$

Using a bit of Planck's old quantum theory, we can set the energy of interaction equal to a frequency (corresponding to the energy of interaction) and therefore calculate the time period of oscillation.

$$E_{ia} = \kappa \mu^2 / n^2 R^3 = \hbar \omega_{int} = \hbar / \tau_{int} \quad , \text{ or }, \quad \tau_{int} = \hbar / E_{ia} = \hbar n^2 R^3 / \kappa \mu^2$$

In other words, the rate of transfer k_T is $k_r = 1/\tau_{int} = \kappa \mu^2 / \hbar n^2 R^3$.

From the theory of a Hertzian oscillating dipole, we know that an isolated quantized Hertzian dipole radiates its energy with a time constant of $\tau_r = 3\hbar c^3 / \omega^3 \mu_D^2$, where ω is the frequency of oscillation of the donor Hertzian oscillator, c is the speed of light.

Now we simply find the distance R_0 where the natural decay time of the oscillator is equal to the time that the energy is transferred, $\tau_r = \tau_{int}$ [23]; this gives

$$R_0^{\ 3} = \frac{3\kappa c^3}{n^2 \omega^3} = \frac{3\kappa}{(2\pi)^3 n^2} \lambda^3 \approx .01\lambda^3 \ ;$$

or $R_0 \approx 0.2\lambda$. So we see that according to this model, the energy transfer would take place over a distance approximately that of $1/5$ the wavelength of light radiating from the oscillating dipole oscillator. This would be about 100 nm, which is much too large.

The reason for the $1/R^3$ dependence, which leads to such a large value of R_0, is the *assumption of exact resonance*. We should be clear what this means. Exact resonance between any two individual molecular oscillators is required in order to conserve energy. However, in an ensemble of molecules in solution, there is a distribution of energies, and the width of this distribution must be taken into account correctly (this was done in gases by Kallmann and London, and by Mensing, Nordheim and Holtsmark, by considering the broadening effects of collisions and Doppler shifts). The critical role that this broadening plays will become clear in our discussion below of Förster's first theory. In the following section I show that this is also the major reason that F. Perrin's quantum mechanical derivation arrived at a distance that was too large. However, we do not want to lose sight of the historical context. The basic model of J. Perrin was correct, and he used all the ideas prevalent at the time: the Maxwellian electromagnetic field of an oscillating Hertzian dipole, the decay constant of a quantized Hertzian dipole (semiclassical description), the Bohr condition of quantum energy jumps and the condition of resonance between the two Hertzian dipoles exchanging energy. When J. Perrin first developed his theory (Perrin, 1925), the two new quantum mechanic theories of Heisenberg (Heisenberg, 1925) and Schrödinger (Schrödinger, 1926b; Schrödinger, 1926a) were just being developed.

1.7.3.2. The Quantum Mechanical Derivation of F. Perrin with Exact Resonance

This section is a résumé of the quantum mechanical theory of F. Perrin (Perrin, 1932; Perrin, 1933). It is beyond the topic of this chapter to go into details, and a full understanding of this section requires some acquaintance with

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[23] The designation R_0 was first given by Kallmann and London in their 1928 publication, and even earlier by Holtsmark and Mensing; the same expression was then used by J. and F. Perrin, as well as Förster.

the quantum theory of two states. But it is important to show his approach, because it is the first detailed quantum mechanical description of energy transfer in solution. He draws on the ideas of Kallmann and London (Kallmann and London, 1928), but he develops a theory applicable for solution studies. He arrived at the same estimate as the classical derivation by J. Perrin; that is, energy transfer over distances of $R_0 \approx 0.2\lambda$, where the wavelength is that of the fluorescence of the donor. This distance is far too long, and the reasons for this are revealing from a historical point of view.

F. Perrin (Perrin, 1933) derived the rate of energy transfer between two identical molecules, each with very narrow energy states. The two molecules are only considered to have two states – the ground state and the excited state – and they are in resonance. A similar derivation was given later by Förster (Förster, 1965a), where he used this theory to illustrate exciton theory with a molecular dimer. Förster mentions that this oscillatory *transfer rate for resonance* between the two monomers of an exciton dimer would be difficult to measure if it took place exactly by this mechanism. Indeed, it is, and to observe optical resonance oscillations requires time resolution far beyond that available in Förster's time. In a normal FRET mechanism – i.e. Förster transfer – the transfer takes place between a single level of the donor to either a continuum, or many closely spaced states, in the acceptor; and the theory for this is different and usually uses the Fermi Golden Rule. The reader must consult F. Perrin's paper for details. Similar accounts of two interacting molecules with two states are available in QM textbooks (Pauling and Wilson, 1935; Davydov, 1965; Landau and Lifshitz, 1965; Schiff, 1968; Cohen-Tannoudji *et al.*, 1977). A very good discussion of many aspects of two-state systems, with and without coupling to the environment, can be found in the textbook by Scully and Zubairy (Scully and Zubairy, 1997). Our short derivation follows Davydov's book (Davydov, 1965), but the derivations of other texts are identical. As mentioned above, the derivation of the basic equations is based on the work of Kallmann and London (Kallmann and London, 1928).

We consider one of the two molecules to be in an excited state, and one to be in the ground state; therefore, the first order perturbation to the energies does not go to zero, as in the London interactions between two ground state molecules (see the section on London's theory). The two wavefunctions (including their time dependence) of the whole system Ψ_1 and Ψ_2 (including both molecules) are sums of products of the stationary states of each of the molecules $\psi(1)$ and $\psi(2)$ (1 and 2 designate the different molecules)

$$\Psi_1 = \frac{1}{\sqrt{2}}\{\psi_n(1)\psi_0(2) + \psi_0(1)\psi_n(2)\}\,e^{-iE_1 t/\hbar}$$

$$\Psi_2 = \frac{1}{\sqrt{2}}\{\psi_n(1)\psi_0(2) - \psi_0(1)\psi_n(2)\}\,e^{-iE_2 t/\hbar}$$

Subscripts n and 0 designate which molecule is in the excited state n or the ground state 0. The molecules are identical, and only one can be in an excited state. E_1 and E_2 are the energies of the two states (formed from the linear combination of the products of the atomic orbitals) for which we have to solve. First order perturbation theory gives the correction to the energies of the combined system (the zero order energy of the two-molecule system, with one molecule in the excited state, is $E_n^0 + E_0^0$) to be

$$\Delta E_1(R) = \frac{e^2}{R^3}\langle \Psi_1 | \hat{D} | \Psi_1 \rangle \ , \ \Delta E_2(R) = \frac{e^2}{R^3}\langle \Psi_2 | \hat{D} | \Psi_2 \rangle \qquad .$$

$\left(e^2/R^3\right)\hat{D}$ is the Coulomb perturbation between the two molecules. Substituting the expressions for the wavefunctions and the perturbation, we get

$$\Delta E_1(R) = -\Delta E_2(R) = \frac{e^2}{R^3}|\langle n | \hat{r} | 0 \rangle|^2 \, \kappa(1,2) \ ,$$

where $|n\rangle$ and $|0\rangle$ designate that the corresponding molecule is in the nth excited state or in the ground state. $\kappa(1,2)$ is the geometric orientation factor between the dipole moments and $|\langle n | \hat{r} | 0 \rangle|^2$ is the square of the dipole transition matrix element. In terms of the oscillator strength of the $0 \to n$ transition, $|\langle n | \hat{r} | 0 \rangle|^2 = \hbar f_{n0}/2\mu_e\omega$ (Förster, 1951; Kauzmann, 1957; Stepanov and Gribkovskii, 1968). This derivation is very similar to the quantum mechanical theory of London's van der Waals forces; however, the London interaction *between two ground state molecules* varies as $1/R^6$ (from the second order perturbation). In the case where *one molecule is in the excited state* and we are at *exact* resonance, the energy of interaction varies as $1/R^3$ (because in this case we must use the first order perturbation). The excitation energy of the two states Ψ_1 and Ψ_2 is distributed at any moment over both molecules; that is, the separate molecules do not have well-defined energies at any time.

Now we calculate the rate of energy exchange between the two molecules according to F. Perrin. Using the calculated energies and wavefunctions, we can write (leaving out all the details) a superposition of the states Ψ_1 and Ψ_2.

$$\Gamma = \frac{1}{\sqrt{2}}[\Psi_1 + \Psi_2] = \{\psi_n(1)\psi_0(2)\cos(vt) + \psi_0(1)\psi_n(2)\sin(vt)\}\,e^{i\left(E_n^0 + E_0^0\right)t/\hbar}$$

$$v = e^2 f_{n0}\kappa(1,2)/\left(2\mu_e\omega R^3\right) \qquad .$$

This is a valid solution to the Schrödinger equation of the whole system. Γ can be substituted into the Schrödinger equation for the combined system, and the coefficients (the time dependent cosine and sine terms) in the above equation can easily be derived. So the time it takes to completely transfer the energy from $1 \rightarrow 2$ (assuming that the energy is solely in molecule "1" at time zero) is

$$\tau = \frac{2\pi}{v} = \frac{\mu_e \pi \omega R^3}{e^2 f_{n0} \kappa(1,2)}.$$

The rate of energy transfer in this case is therefore

$$k_{ET} = \frac{1}{\tau} = \frac{e^2 f_{n0} \kappa(1,2)}{\mu_e \pi \omega R^3} = \frac{4}{h} \frac{e^2}{R^3} \left|\langle n|\hat{r}|0\rangle\right|^2 \kappa(1,2) = \frac{4}{h}\left|\Delta E_1(R)\right|$$

We see that the rate of transfer is proportional to the energy splitting of the two exciton energy levels $\frac{2}{h}\left|\Delta E_1(R)\right|$, which is proportional to the square of the transition moment $\left|\langle n|\hat{r}|0\rangle\right|^2$. F. Perrin's quantum rate of transfer of energy shows the same $1/R^3$ dependence as J. Perrin's classical derivation. Again, the reason is that we have assumed exact resonance of the two oscillators at all times, and infinitely sharp energy levels have been assumed. In addition he chose to look at the dynamics of the oscillating term, which Kallmann and London did not. If we use the well known expression for the Einstein rate coefficient in terms of the oscillator strength, which is the natural rate of fluorescence emission k_e competing with the energy transfer, and set the rate of emission equal to the rate of energy transfer derived by F. Perrin, $k_e = k_{ET}$, we can calculate the distance where half the energy will be transferred within the excited state lifetime. This gives $R_0 = 0.19\lambda$, which is again the same as was obtained from the calculation of two classical oscillators in exact resonance; this is much too large to explain the distances of interaction measured in solution. F. Perrin calculates $R_0 = 0.22\lambda$. F. Perrin was aware that this distance was too large, and he suspected that broadening of the spectra could lead to shorter R_0 distances. As we learned in the section "F. Perrin's Model", he invoked collisional broadening (by the solvent), which did decrease R_0 to approximately 25 nm. This was still much too long. We now know that collisional and Doppler broadening is miniscule compared to broadening caused by specific interactions with the solvent (such as polar effects).

The oscillation of this system between two interacting two-state molecules with sharp energy levels, where the two systems are in exact resonance, behaves

essentially the same as what is known as *Rabi oscillators*. Such resonant oscillators are named after Rabi (Rabi, 1936; Rabi, 1937) and were originally obtained by Güttinger (Güttinger, 1931) to describe a spin subjected to a time-dependent magnetic field. In the optical realm, this is called an optical Rabi oscillation (Cohen-Tannoudji *et al.*, 1977; Allen and Eberly, 1987; Loudon, 2000). The oscillatory exchange between the two states of an atom in resonance with an optical field (photons) near the resonance frequency is called optical nutation (taking over the terminology of spin nutation in magnetic resonance). The frequency is essentially the interaction energy divided by h, $(4/h)|\Delta E_i(R)|$. These are the solutions for the Bloch equations (Bloch, 1946) describing the time dependent interaction of a two state quantum system with a weak perturbation of an oscillating electromagnetic field in exact resonance with the molecular system (Allen and Eberly, 1987). In the case of an externally applied optical field (light), the oscillatory energy exchange is between the two-state molecule and the electromagnetic field.

In F. Perrin's case the electromagnetic interaction is from the near field of the other identical molecule, and the energy exchange is between the two molecules. In general, if multiple discreet eigenstates are well isolated from all the other levels of the unperturbed Hamiltonian, then the transitions between the two levels are superpositions of Rabi oscillators. When the number of coupled states becomes very large (which is the normal case for fluorophores in solution) the different Rabi oscillations with different frequencies and amplitudes interfere, and then the system evolves with the normal irreversible character.

The important point is that the interacting molecules of F. Perrin were limited to only two states, and the two molecular oscillators were exactly the same and in exact resonance. The result is valid for the case of identical molecules where the width in energy levels of the two states, $\psi_n(1)\psi_0(2)$ and $\psi_0(1)\psi_n(2)$ are less than $2|\Delta E_i|$, which is the energy splitting caused by the perturbation. This results in a coherent interaction, and that is responsible for the prediction of the FRET interaction at much longer distances. However, if coherence is not lost during the interaction between two atoms or molecules with discreet eigenstates, the distance dependence of the rate of energy transfer would be $1/R^3$, as calculated by J. and F. Perrin. This would happen, for instance, in a vacuum between two isolated atoms. This is not an irreversible transfer of energy from a donor to an acceptor, because in this idealized case (for solution) there is an oscillatory "back and forth" transfer. See two papers by Robinson and Frosh(Robinson and Frosch, 1962; Robinson and Frosch, 1963) and references there-in for more detailed discussion of the introduction of irreversibility with multiple states, which leads to the normal exponential decay,. If the perturbation of the two state system is not resonant, a more involved calculation shows that the probability for complete transfer is less than one. But the system still oscillates, and still shows the $1/R^3$ dependence of the oscillation frequency (Cohen-Tannoudji *et al.*, 1977).

1.7.4. The Contribution of W. Arnold and J.R. Oppenheimer to FRET in Photosynthesis

It is not commonly known that Oppenheimer reported the theory of FRET (with energy transfer at the correct distances) in 1941 (Oppenheimer, 1941). Perhaps even less well known (except in the field of photosynthesis) is that Oppenheimer's contribution to FRET, together with Arnold, led to a major advance in our understanding of photosynthesis. Although the full description of Arnold and Oppenheimer's contribution was not published until 1950 (Arnold and Oppenheimer, 1950) (probably because of his work in Los Alamos; in 1950 he was at the Advanced Institute at Princeton). Even though this latter publication is after the contributions of Förster (Oppenheimer's earlier abstract was not known by Förster at the time he developed his theory) it is clear that Oppenheimer had the correct solution in 1941; so I will consider this first.

1.7.4.1. Oppenheimer's Short Abstract of 1941

At the American Physical Society in 1941, a paper was presented by J. R. Oppenheimer, entitled "Internal Conversion in Photosynthesis" (Oppenheimer, 1941). As we know, Arnold had gone to Oppenheimer in 1940 consult about this problem (Arnold, 1991), and this short abstract was the result of their work together. Dutton wrote a historical account of sensitized photosynthesis, with a discussion of Arnold and Oppenheimer's contributions (Dutton, 1997)). In order to account for the rate of photosynthesis when light was absorbed by certain dyes (which absorbed where chlorophyll absorbed little), the energy must be transferred to chlorophyll, where it can then be transported to the photosynthetic reaction sites. In the abstract (Oppenheimer, 1941) Oppenheimer points out that the high efficiency of this transport of energy cannot be due to light emission and re-absorption (the probability for this is too small). However, the energy transfer could be enhanced if the chlorophyll molecules are much closer than the wavelength of the chlorophyll fluorescence (near field of a Hertzian dipole). In this abstract, the ratio of the number of quanta transferred to the number of quanta emitted as fluorescence is given as $n\sigma\lambda^4/d^3$, where d is the closest distance of approach between the chlorophyll molecules, n is the chlorophyll concentration, σ is the absorption coefficient, and $2\pi\lambda$ is the wavelength of light in water. This is for the case of chlorophyll molecules located randomly in space, and he has already integrated the rate of energy transfer over all molecules from d to infinity (see the next section). Although in this abstract Oppenheimer does not show the solution for the rate of transfer between only two molecules, the $1/d^3$ result is obviously the result of integrating $1/r^6$ from d to infinity. So it is clear that he had the correct equation. He also assumed a quantum yield of one for isolated chlorophyll molecules.

There is a very interesting sentence in this abstract: "This transfer gives a large scale model of the internal conversion of nuclear gamma-rays.". Oppenheimer was well versed in the theory of *internal conversion* in nuclear physics - the non-radiative transfer of energy between a radioactive nucleus and tightly bound electrons, which is a process that competes with the emission of gamma rays. Very probably Oppenheimer already had the solution applicable to FRET at his fingertips. Arnold and Oppenheimer discuss this in more detail in their subsequent 1950 paper (Arnold and Oppenheimer, 1950). I will explain the remarkable analogy between FRET and nuclear internal conversion in the next section.

It is not surprising that this abstract was not noticed by many researchers, and certainly not by those interested in chemical and biological systems. The reasons are many: the war, the shortness of the abstract, the audience where it was presented, and the fact that he did not present a general expression for energy transfer – he only presented the integration over the distribution of acceptors that was relevant specifically to his particular problem. He became interested in this topic through his contact with William Arnold, an expert and pioneer in photosynthesis (Knox, 1996). There is no record of Oppenheimer's talk, other than this abstract, and I have not found anyone who heard Oppenheimer's presentation. At this time he was in the California Institute of Technology and Berkeley, and had not yet started to work on the war effort in Los Alamos. In an article by Arnold (Arnold, 1991), he recounts how Oppenheimer came to know about the photosynthesis problem[24]. Emerson had told Arnold of the experiments indicating the transfer of energy from phycocyanin to chlorophyll. Arnold did some experiments to verify this, and then went to Berkeley in 1940 where Oppenheimer was at the time, and consulted with him about the apparent energy transfer. As soon as Oppenheimer heard about the problem, he realized the analogy with internal conversion in radioactive nuclei, and the connection to gamma rays – only the length scale was different by 10^4 (see the end of the next section). Arnold had known Oppenheimer since 1935 when he went to Berkley to audit Oppenheimer's course on quantum mechanics. We now turn our attention to the later paper by Arnold and Oppenheimer (A&O) from 1950 (Arnold and Oppenheimer, 1950).

1.7.4.2. Arnold and Oppenheimer's Derivation of the Rate of Energy Transfer of 1950.

In 1950, A&O published the work (Arnold and Oppenheimer, 1950) that was alluded to in the abstract of 1941[25]. In this paper, they provided a mechanism of energy transfer from phycocyanin (which is one of the accessory dyes in plants, in addition to e.g. carotene, xanthophylls and phycoerythrin) to chlorophyll in the blue green algae. The major question was "whether or not any of the light energy absorbed by these accessory pigments is used by the

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[24] I thank Robert S. Knox for reminding me of this history.
[25] This was Oppenheimer's last official scientific publication.

plant to reduce carbon dioxide" (all quotes in this section are from the A&O paper). It had been shown by Emerson and Lewis (Emerson and Lewis, 1942) that almost all of the energy absorbed by phycocyanin in *Chroococus* is used with an efficiency of approximately one in photosynthesis; the efficiency of photosynthesis was essentially as high as if the photons had been absorbed by chlorophyll. For our purposes we concentrate on A&O's contributions to the theory for energy transfer.

A&O consider three ways the energy could be transferred: 1) by direct collision, 2) by trivial emission and reabsorption of fluorescence by another molecule, and 3) by "internal conversion, or the resonance transfer of energy from one oscillator to another in resonance with it, and lying within the quasistatic rather than the wave zone field of the former". Direct collision only happens for distances on the order of atomic dimensions, and does not require resonance; in addition, the concentration of acceptors is too small, their mobility is highly restricted and the spatial separation is much too great for collisions to occur in the excited state lifetime. Trivial absorption-reabsorption happens only for distances larger than the wavelength of the emitted light, and in the far-field zone. In this case, they calculate the percentage of light transferred is only roughly $F\sigma nR = 10^{-3}$ which is much too small (F is the fluorescence quantum yield, σ is the absorption cross section of the dye, n is the concentration of chlorophyll – the acceptor – and R is the dimension of the cell). Therefore, they consider transfer in the near field of Hertzian dipole radiation, for which "the electric field of an oscillator (emitting primarily electric dipole radiation), which in the wave zone falls off linearly with the inverse distance from the emitter, increases, as the emitter approaches within distances small compared to a wave length, as the inverse cube of the distance". He also makes the important point, which is often not appreciated, that the energy transfer efficiency can be much greater that the fluorescence efficiency if the two molecules are close enough. This was very important for photosynthesis because it was known that the *in vitro* fluorescence quantum efficiency of the accessory pigments was often much smaller than the quantum efficiency of the energy that was transferred to the photosynthetic unit.

A&O then embark on a succinct two page derivation of the rate of energy transfer. We cannot give this derivation justice, but only skim the important points for this FRET history. The reader is referred to their paper (which is terse; it is not particularly easy to read unless you are versed in EM calculations). First they calculate the fluorescence yield of the Hertzian dipole by considering the Poynting vector, using the EM vector and scalar potentials (Greiner, 1986) at large distances (far field zone) from the donor oscillator; then they calculate the rate of absorption of the acceptor dipole that is in the near field zone of the donor oscillator, using again the vector and scalar potential of a Hertzian dipole, and using the expression for the absorption coefficient in terms of the electric transition dipole moment $\vec{M}$.

The electric field in this near zone is (keeping his notation)

$$\vec{E}_a = \frac{2C_1 \sin 2\pi\nu t}{2\pi\nu r^3}\left[\vec{a} - \frac{3(\vec{a}\cdot\vec{r})\vec{r}}{r^2}\right] \qquad .$$

$\vec{a}$ is the amplitude of the vector potential $\vec{A}$ oscillation[26], C_1 is the speed of light in the medium (water), ν is the (spectroscopic) oscillator frequency, r is the distance from the oscillator to an acceptor, and h is Planck's constant. For some readers, the vector potential may be unfamiliar – it is a potential used in EM calculations with Maxwell equations (actually first introduced by Maxwell), which is an analogue of the scalar electric potential $\phi(\vec{r})$ (which Oppenheimer also uses)[27]. The expression in square brackets represents the orientation factor (the direction of the E-field in vector notation).

The rate of absorption of an acceptor molecule in the near zone of the donor E-field is calculated as

$$\left\{C_1^2\vec{M}^2\left[\vec{a}^2 + 3(\vec{a}\cdot\vec{r})^2/r^2\right]\right\}\Big/3h^2\nu^2 r^6 \quad .$$

$\vec{M}^2$ is the square of the transition dipole moment of the acceptor. Here Arnold and Oppenheimer have assumed that the acceptor molecule, bathed in the EM field of the near zone of the donor Hertzian dipole, will absorb energy according to the same rate equations (Fermi's Golden Rule) as if the acceptor molecules were bathed in the EM field of radiation (light). The expression in square brackets is $\vec{a}^2$ times the usual κ^2 orientation factor of FRET. This rate of energy transfer is the same as derived by Förster (see below), but in terms of the vector potential squared and it has been assumed that the fluorescence quantum yield of the donor is one (you just have to multiply the equation by the quantum yield). The equation for the rate of energy transfer (which requires knowing an expression for $\vec{a}$) is to be compared to the expression of Förster (see later).

Next, they average over all angles, giving the following equation for the rate of energy transfer to a randomly oriented acceptor at a distance of r (this averages the orientational factor, to give $\kappa^2 = 2/3$).

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[26] The oscillating vector potential of a Hertzian dipole at position r is

$$\vec{A} = (2\vec{a}/r)\cos 2\pi\nu\,(t - r/C_1)$$

[27] As a reminder for those who are familiar: the magnetic field in terms of the vector potential $\vec{A}$ is

$\vec{B} = \nabla \times \vec{A}$, and the electric field in terms of the scalar potential ϕ and the vector potential is

$$\vec{E} = -\nabla\phi - \partial\vec{A}/\partial t \quad .$$

$$T(r) = \frac{\sigma \vec{a}^2 C_1^3}{4\pi^3 h v^3 r^6}$$

They have substituted the expression $\vec{M}^2 = 3hC_1\sigma / 8\pi^3 v$, where σ is the absorption cross section of the acceptor.

Finally they integrate over a uniform distribution of acceptor oscillators, with a density of n outside a sphere, surrounding the donor with a radius of d, which is the radius of closest approach.

$$T = \frac{\sigma \vec{a}^2 C_1^3 n}{3\pi^2 h v^3 d^3}$$

This is the same rate of energy transfer given in Oppenheimer's abstract of 1941 (Oppenheimer, 1941), just in terms of other variables. Then A&O go on to calculate expectations from this theory and compare to experiments. Of course, when comparing to experimental data the overlap of the energy levels, the spectral distributions of the donor and acceptor must be taken into account (the overlap integral). I remind the reader of our discussion of Holtsmark's publication, where he arrived at a very similar equation with the same definition of (and dependence on) d (Holtsmark, 1925).

I now calculate some quantities that were not included in their publication. Substituting their expression for $\vec{M}^2$ into the expression for $T(r)$ above (which is the rate of energy transfer to one acceptor at a distance of r), and setting this equal to the rate of emission from the Hertzian dipole

$$T_{em} = \frac{16\pi^2 v \vec{a}^2}{3hC_1} \quad ,$$

and assuming that the orientation factor is $2/3$, we can calculate

$$R_0^6 = \frac{3}{64} \frac{C_1^4}{\pi^5} \left(\frac{\sigma}{v^4} \right) \qquad \text{; this assumes that the quantum yield } q = 1.$$

The absorption cross section and quantum yield are in fact dispersed over a spectrum of frequencies; that is, $\sigma(v)$ and $q(v)$. And the quantum yield $\int q(v) dv$ is not usually 1. To account for the spectral spread of the donor and acceptor spectra, we have to integrate over the total frequency interval. We also

remember that $C_1 = c/n$, where c is the speed of light in a vacuum and n is the index of refraction pertaining to energy transfer. This gives

$$R_0^6 = \frac{3}{64}\left[\frac{3}{2}\kappa^2\right]\frac{c^4}{\pi^5 n^4}\int\frac{\sigma(v)q(v)}{v^4}dv = \frac{9000\ell n10}{128}\frac{\kappa^2 c^4}{\pi^5 n^4 N_A}\int\frac{\varepsilon_A(v)q_D(v)}{v^4}dv.$$

To arrive at the last equality I have simply substituted the molar decadic molar absorption coefficient of the acceptor $\varepsilon_A(v)$ and the fractional quantum yield factor $q_D(v)$. N_A is Avogadro's constant. This last expression is exactly the expression for R_0 that was derived by Förster (see below). I have put the orientation factor, κ^2 back in both expressions. A&O did not carry this simple calculation for R_0 through, and they did not consider the overlap integral, because they were interested in calculating the expression for the rate of transfer to all the acceptors located randomly, which is the expression for $T \propto 1/d^3$ given above. But this shows that their derivation gives the same result as Förster's.

Although we have skipped over details of the derivation (which A&O also did not furnish) it may have occurred to the reader that this derivation did not explicitly use a spectral distribution of frequencies of the donor and acceptor (until we added it at the end in an *ad hoc* manner). Why then, did the problem that was encountered by Perrin (a $1/R^3$ behavior) not happen here too? It might seem as if we are again dealing with a coherent interaction of two oscillators with identical single frequencies. The reason is subtle, but very important. In effect, the oscillators have already been considered to be incoherent, and an integration over the spectral distribution of acceptor energy levels has already been carried out, because he used the solution-phase absorption coefficient. That is, it is not a two-state system. When calculating quantum rates (e.g. rates of absorption and emission) resulting from time-dependent perturbation theory, where the perturbation and the perturbed quantum system are considered to have coherence times short compared to the interaction, one usually uses the Fermi Golden Rule. This rule is derived by taking into account the distribution of available quantum states (in the molecule, and/or in the field) by integrating over the frequency distribution. This is the same for Förster's theory. The spectral distribution is very important, but can be introduced in different ways. Oppenheimer was well aware of this. By 1941-1950, quantum mechanics had been refined a great deal after F. Perrin developed his quantum mechanical theory of energy transfer (Perrin, 1932; Perrin, 1933).

In this paper, Oppenheimer refers several times to the identical theoretical treatment of energy transfer and "the process of internal conversion that we have in the study of radioactivity". Because this is a remarkable historical

connection between nuclear physics and fluorescence spectroscopy, which has apparently not been recognized very often, I discuss this mechanism shortly so the parallel is clear[28].

An excited nucleus (e.g. a radioactive substance) can undergo spontaneous emission of a photon (a gamma ray). Depending on the nuclear levels, as the nucleus undergoes a transition from one level to another one speaks of the emission of a photon, a gamma ray, of a particular multipole transition. However, this nuclear transition can also take place by transferring energy directly to one of the orbital atomic electrons (e.g. K electrons) by a *non-radiative* mechanism. The atomic electron absorbs the energy non-radiatively, and is subsequently ejected through the strong interaction of the electron and the nuclear currents and charges via the large electromagnetic field in the near zone. This is named *internal conversion* of the electric or magnetic multipole. The ratio of the rate of electron emission W_e to the rate of gamma-ray emission W_γ is defined as the *internal-conversion coefficient,* $\alpha = W_\gamma / W_e$, and there are extensive tables of these coefficients. An identical ratio is what Oppenheimer calculated for FRET in this publication with Arnold. The dipole field of the nuclear transition is the same as that which is considered for FRET, only on a much smaller scale. Especially for the heavier atoms, the electrons have a relatively large probability of being very close to the nucleus, where the electron interacts with the near field of the nucleus. This interaction varies as $1/R^3$, and the rate of internal conversion in the near field zone varies as $1/R^6$, just as for FRET. This near field effect increases the probability of transfer (internal conversion) dramatically – just as in FRET. Actually, the theory of this nuclear internal conversion is *identical* to FRET, except for the scale. The rate expression, in terms of the square of the dipole transition moments, is identical. The internal conversion electron spectra (observing the properties of the ejected electrons) are similar to observing the fluorescence of the acceptor in FRET. The observation of the gamma rays is similar to observing the decreased emission of the donor in the presence of the acceptor. I will not go into this interesting comparison further, or all the information concerning the electron and nuclear states that can be derived (Siegbahn, 1965). The important historical point is that Oppenheimer realized these similarities; he had worked extensively with the theory of internal conversion in the nucleus, and he also realized that the theory of internal conversion could be applied to energy transfer in photosynthesis.

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[28] I thank Hans Frauenfelder, who gave me the initial literature reference for researching nuclear internal conversion.

1.8. FÖRSTER'S SEMINAL CONTRIBUTION: THE MODERN, PRACTICAL DEPICTION OF FRET (*FÖRSTER* RESONANCE ENERGY TRANSFER)

This brings us to the numerous contributions of Theodor Förster, which is the culmination and end point of our history. Förster's theory and the accompanying experimental work on energy transfer is the most widely known, and most influential, of all FRET publications. The major papers are listed here (Förster, 1946; Förster, 1947; Förster, 1948; Förster, 1949a; Förster, 1949b; Förster, 1951; Förster, 1959; Förster, 1960; Förster, 1965b; Förster, 1965a; Förster, 1993). The 1993 reference (Förster, 1993) is an English translation by Robert S. Knox of Förster 's 1948 paper. Förster provided an accessible theory in a form that was practical for experimenters. It is difficult to exaggerate the influence of his work. His papers are still referenced in every paper dealing with FRET. His famous book (Förster, 1951) has also been cited thousands of times, but I suspect, since the book still exists only in German, it is seldom read, which is unfortunate because it is excellent. Regrettably, several of his papers have never been officially translated; however, there are some excellent translations of critical papers; for instance (Förster, 1993). One interesting later English paper is (Förster, 1960). His influence is remarkable, considering that he may just hold a record of citations that have not been read, or even seen, by many authors. Because the literature discussing Förster's contributions is extensive, I will not dwell on details. But this should not diminish the fact that the extensive, widespread use of FRET in physics, engineering, chemistry, biology and medicine, are due to the description of energy transfer given to us by Förster. The following account is meant to highlight why this is so.

Förster apparently became interested in the energy transfer process because of the known effectiveness of photosynthesis (just as Oppenheimer); although, he was also aware of the work of the Perrins. Experiments (Emerson and Arnold, 1932a; Emerson and Arnold, 1932b) had shown that the capture and utilization of the light energy by plant leaves was much more effective than would be expected if it were required that photons exactly hit the reaction centers (that is, there are too few reaction centers in the leaves to explain the very effective extent of energy capture). Förster knew of these results, and reasoned that an extremely efficient transfer of energy between the chlorophyll molecules must be responsible for the eventual diffusion of the energy, which was absorbed over the whole surface of the leaf, into the relatively sparse reaction centers. He assumed that this energy diffusion is due to energy rapidly hopping between molecules. He was also aware of the earlier work of the Perrins, and of other data indicating energy transfer over distances longer than the molecular diameters. He gives a thorough account of this early work in his initial papers.

In his first paper on FRET (Förster, 1946), he correctly developed the basic theoretical background of FRET (an English account of this derivation has been given (Clegg, 1996)). First he reviewed the mechanisms proposed by the Perrins. He then proceeded to take three critically important steps that allowed

him to derive a quantitative theory of non-radiative energy transfer (Förster, 1948). The reader should keep in mind our discussion of A&O's work and that of J. and F. Perrin, while reading the following. Förster did not know of Arnold and Oppenheimer's derivation, I have included information there that will not be repeated here.

1) Förster was well versed in the new quantum theory describing the electronic structure of molecules. He knew that the atomic vibrations in complex molecules and interactions with the solvent in condensed media considerably broaden absorption and emission spectra. These theories of broad condensed system spectra had been developed mainly subsequently to the original work of the Perrins. The theoretical quantum mechanical treatments of spectroscopic transitions had clearly shown the necessity of taking into account the effect of broadened energy distributions when calculating the rate of a kinetic process between two quantum states (Dirac, 1927). This leads to the famous *Fermi Golden Rule*, which quantitatively relates the rate of transition between states of a quantum molecular system that is perturbed by an oscillating electromagnetic field, such as a light wave[29]. In a similar manner, Förster took into account the broad spectral dispersion of the donor fluorescence and acceptor absorption. The actual experimentally determined spectral breadths correspond to much broader energy dispersions than that calculated by F. Perrin from collisions, or from the spectra in vapors. This significantly affects the estimates of the probability that the frequencies (energy differences between the excited and ground states) of the donor and acceptor molecules will be simultaneously nearly identical. See our discussion of this in the section "F. Perrin's Model". He correctly took into account the overlapping oscillation frequencies of the donors in the excited state and the acceptor molecules in the ground state. In his first paper (Förster, 1946), Förster treated this frequency overlap semi-classically and semi-quantitatively. Shortly thereafter (Förster, 1947; Förster, 1948) he gave a full quantum treatment. He showed how to express this overlap quantitatively in terms of the frequency dependent "oscillator strengths" of the classical or quantum spectroscopic transition dipoles (Förster, 1951). The frequency dependence of oscillator strengths of a spectroscopic transition is a theoretical way to represent the shape of the measured spectroscopic spectra. The stringent requirement that the two molecules be in resonance to effect energy transfer, is the reason that Förster emphasized the name "resonance". Of course, the requirement for resonance had been emphasized by all the previous theories, classical and quantum mechanical.

2) Förster realized that the classical theory of interacting oscillating dipoles, which he had shown could lead to an exchange of energy between molecules, is very similar to the interaction of a single molecular transition

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[29] The Fermi Golden Rule was actually derived by Dirac in 1927.The common attribution of the golden rule to Enrico Fermi is misleading; it was Dirac who developed time-dependent perturbation theory, including this formula. It was coined Fermi's Golden Rule because in Fermi's famous lectures in Chicago he used the rate expression, and called it the golden rule (of course, he did not call it Fermi's Golden Rule). Although Fermi never claimed, or suggested, any priority, his name has remained associated with this rate expression.

dipole with the oscillating electric field of light, which theoretically describes spectroscopic transitions of absorption and fluorescence. Thus he was able to develop a quantitative theory (see the equations below) of the rate of transfer from an excited donor molecule to a ground state acceptor molecule in terms of what is now known as the *overlap integral*. We have seen that A&O applied the same reasoning (which was also applied in the internal conversion of nuclear transitions). However, A&O's rate expression does not present the integrated overlap of the absorption and emission spectra, and they do not give an explicit expression for the orientation factor (that is, they leave it up to the reader to derive this from the vector expression). A&O were not interested in general applications of FRET; they were solving a particular problem; although their equations are quite general and are identical to Förster's. Förster explicitly deals with the vibrationally broadened spectra and he derives the overlap integral in terms of the measured absorption and fluorescence spectra. Förster also gives helpful expressions for the orientation factor, and discusses κ^2 (and introduces this notation). The overlap integral is the integral of the product of the donor fluorescence spectrum and the acceptor absorption spectrum over the entire frequency range, divided by v^4. I will not go into the factor v^4, but it also arises in the expression for the rate of transfer I have given in the discussion of Arnold and Oppenheimer's work[30]. The overlap integral represents the probability that the two molecular transition dipoles will have the same frequency. This was a major conceptual step, because *these spectroscopic transitions can be measured experimentally*, independent of the FRET measurement. It is also important to realize that no "spectroscopic transition" takes place; that is, there is neither the emission of a photon nor the absorption of a photon in the FRET process. Fluorescence comes into the picture because the method normally used to measure FRET is fluorescence; it is not part of the actual physical process. It just happens that the electromagnetic interaction between the donor and acceptor can be described in terms of the same theoretical expression as the normal absorption and emission of a photon[31]. We should keep in mind that the earlier work of Mensing, Holtsmark, Nordheim, Kallmann & London, the Perrins, and Arnold and Oppenheimer also realized that the dipole-dipole interactions took place over these spectroscopic transition moments. However, Förster showed unambiguously how to connect this overlap to measured absorption and emission spectra, and gave an explicit expression for the overlap integral, opening the way to quantitative interpretation of experimental data. He included the effect of the index of refraction, which affects all electric interactions in condensed media at these very high optical frequencies, $\approx 10^{15}$ sec^{-1}. The relative orientation of the two dipoles will control the strength of their interaction at a certain distance. This is the infamous *kappa square*, which has led to great discussion in the literature

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[30] This requires a bit of calculation: σ has a factor of v, and $\bar{a}$ has a factor of v^{-1}.

[31] It is the perturbation of an oscillating E-field (of the transition moment) interacting with the electronic ground state of a molecule.

and is one of the major complications of FRET interpretations. The quantum yield of the donor fluorescence, or the fluorescence lifetime, in the absence of the acceptor, appear because these parameters are related to the strength of the oscillating dipole (classical model) or the transition dipole (quantum model).

3) Förster's model included quantitatively the $1/R^6$ distance dependence of the dipole-dipole interaction. This corrected the $1/R^3$ dependence of the Perrins' solutions for energy transfer in solution. Similar to previous theoretical accounts, Förster calculated the distance R_0 where the rate of the energy transfer was equal to the rate of fluorescence emission. This distance is now universally known as R_0 [32]. He showed how R_0 can be calculated from the overlap integral, the quantum yield of the acceptor, the lifetime of the donor in the absence of an acceptor and the effective index of refraction, resulting in a very concise expression for the distance dependence of FRET efficiency (see the equations below).

His resulting famous equations for the rate of energy transfer and the efficiency are (Förster, 1948; Förster, 1951; Ketskeméty, 1962) [33]:

$$\text{rate}_{\text{energy transfer}} \equiv k_{ET} = \frac{9000 \ell n 10}{128 \pi^5} \frac{\kappa^2 c^4}{n^4 N_A \tau_e R^6} \int_0^\infty f_Q^D(v) \varepsilon^A(v) \frac{dv}{v^4} = \frac{1}{\tau_e} \left(\frac{R_0}{R} \right)^6$$

$$\text{Efficiency of transfer} \equiv E = \frac{k_{ET}}{k_{ET} + 1/\tau_e} = \frac{1}{\left(R/R_0 \right)^6 + 1}$$

$\int_0^\infty f^D(v) \varepsilon^A(v) dv / v^4$ is the overlap integral of the normalized fluorescence spectrum of the donor and the extinction coefficient of the acceptor, κ^2 is the kappa square factor that takes into account the relative orientation of the two transition dipoles, N_A is Avogadro's constant, n is the refractive index of the environment of the donor and acceptor, c is the speed of light, τ_e is the

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[32] The terminology R_u, or r_0, was introduced already in the very early papers of Mensing and Holtsmark.

[33] Förster's equation for the rate of energy transfer was first published with a printing mistake; but one that seemed to plague several of his following publications, and even in his 1951 book this mistake persisted (there was apparently one printing of the book where this factor was correct).

This was the π^5 factor in this equation, which was printed in several of his publications as π^6. The mistake was first noticed by Ketskeméty, who in 1962 had offered a different fully classical derivation. Ketskeméty's correction was acknowledged by Förster. It was undoubtedly a printing error, and is well known; but it is interesting that this error persisted even into much later works of Förster, (for instance, Förster, 1959, 1960). It was given correctly in Förster, 1965.

average dwell time of the donor in the excited state in the absence of the acceptor (this is the same time as the fluorescence lifetime), and R is the distance between the centers of the two molecular transition dipoles. R_0 is, as before, the distance between the two fluorophores where the rate of energy transfer is equal to the rate of fluorescence of the donor without the presence of the acceptor. These same equations were given in a short historical account of FRET, which had a few misprints.[34]. Regarding the equation for the efficiency E, it is interesting to note, as mentioned above in the section on Kallmann and London's work, that Kallmann and London wrote this equation in the process of their derivation equation 17 of their paper (Kallmann and London, 1928)).

Förster's theory is the basis of our present understanding of FRET and most of its applications. There have been several extensions of the theory to other experimental conditions, also by Förster himself; however, within the validity of his model, which encompasses most of the applications, Förster's original theory still applies. Förster's original theoretical description of energy transfer set the stage for all subsequent applications of FRET in many fields of research (from pure physics to biology), and it is his theory that still is used to interpret experimental results. He extended the original ideas of the Perrins involving the well known interaction of molecular dipoles; however, his insight and great contribution was to provide the quantitatively correct and very practical description of the FRET process *in terms of experimentally accessible parameters*. By relating the rate of energy transfer to purely experimentally available parameters (except for the kappa square term, which can usually be estimated; there is an extensive and still hotly debated literature dealing with this factor), he provided the general theoretical framework for all FRET applications. FRET has been shown to be broadly applicable and extremely informative for determining molecular interactions, and to measure molecular distances that are impossible to determine otherwise.

1.9. MATURATION OF FRET

Since the seminal papers by Förster, there has literally been a flood of papers, theoretical and experimental, dealing with FRET. Although Kallmann and London, and J. and F. Perrin, as well as Mensing, Nordheim and Holtsmark, set the stage for the correct interpretation for FRET, Förster furnished the clear and explicit connection to experiment. As in all science endeavors, once a theory is developed that can easily be compared to experiment, this opens the door to wide-ranging experimentation in diverse fields.

Extensive Russian literature, much of it unavailable to non-Russian readers, contributed to the theory of energy transfer and to fluorescence in general. I

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[34] In this earlier paper the exponents that are supposed to be to the 6th power were mistakenly given as the 1/6th power, and c^2 was given as e^2 ; and the 9 should be 9000.

have not mentioned these contributions, although Förster mentioned some of the early work in his first papers. Most references to this work can be found in an English book by Agranovich and Galanin (Agranovich and Galanin, 1982); the authors have included a short history of energy transfer. Also, many references to work following Förster can be found in a recent very readable book on FRET (Van Der Meer *et al.*, 1994). Another review (Clegg, 1996) also has many references to much of this literature. The chapters of a recent book edited by Andrews and Demidov (Andrews and Demidov, 1999) have accounts of more recent advances, with many references. I apologize to the many authors who have made vital contributors to FRET who have not been explicitly mentioned; this is simply because of lack of space, as well as the time-frame of this history.

1.10. EPILOGUE

Thus, our history of FRET closes with Förster. We have covered the major contributions to the ideas of energy transfer leading up to Förster. There have been many critical contributions since Förster's first papers. The history following Förster's early work (and also contributed by Förster) is extensive (actually enormous) and rich in innovative experimentation and theory. But this will have to await another chapter of FRET history.

A final point: This chapter has covered mostly work previous to Förster, leading up to his final, practical expression for FRET. Förster always gave explicit reference to the pioneers who preceded him. Yet I would recommend restricting the acronym FRET to *Förster Resonance Energy Transfer*. Förster put all the pieces together. His theory has been tested thoroughly, and if the conditions for which his derivation is applicable are met, his theory has always been found to be valid. There are other modes of energy transfer, and circumstances where Förster transfer is not valid; these require different theoretical foundations. However, reserving "Förster" for the "F" in FRET, whenever we mean Förster transfer, gives credit to the person who made it possible for us to gain valuable, quantitative insight into so many processes at the molecular scale, through relatively easy experiments.

1.11. ACKNOWLEDGEMENTS

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