Extended Förster Theory of Electronic Energy Transport within Pairs of Reorienting Chromophoric Molecules

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Akademisk avhandling

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An extended Förster theory (EFT), previously derived (L. B.-Å. Johansson et al. J. Chem. Phys., 1996,105) has theoretically been adapted and used in simulations of donor-acceptor energy transfer (DAET), which is a process often referred to as FRET. It was shown that the classical Förster theory is only valid in the initial part of the fluorescence decay.

In this thesis an EFT is derived and outlined for electronic energy transport between two fluorescent molecules which are chemically identical, but photophysically non-identical. The energy migration within such asymmetric pairs is partially reversible and therefore referred to as partial donor-donor energy migration (PDDEM). The previously derived model of PDDEM (S. V. Kalinin et al. Spectrochim Acta Part A, 2002,58) is an approximation of the EFT. In particular, the EFT accounts for the time-dependent reorientations as well as the distance that influence the rate of electronic energy migration. The reorientation of the fluorophores transition dipole moments has been simulated using Brownian dynamics. As a result, the related “χ^2 problem” has been solved. The EFT of PDDEM has also been studied regarding the effect of PDDEM on experimental observables e.g. quantum yield of fluorescence and steady-state anisotropies.

Keywords
- electronic energy migration/transfer
- extended Förster theory
- orientation factor
- partial donor-donor energy migration (PDDEM)
- donor-donor energy migration (DDEM)
- fluorescence relaxation
- time-resolved fluorescence anisotropy
- time-correlated single photon counting
- distance measurements
- protein structure
- Brownian dynamics (BD)

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This thesis is based on the following papers, which are referred to in the text by the Roman numerals I-III. The papers can be found as reprints at the end of the thesis, with kind permission by the publishers.


*On the quantitative molecular analysis of electronic energy transfer within donor-acceptor pairs*


II  N. Norlin, P. Håkansson, P.-O. Westlund and L. B.-Å. Johansson

*Extended Förster theory for determining intraprotein distances; Part III. Partial donor–donor energy migration among reorienting fluorophores*


*Fluorescence Spectroscopic Properties Analysed within the Extended Förster Theory with Application to Biomacromolecular Systems*

J. Fluorescence, (2009), in press
1. Introduction

The first observations of the phenomenon of fluorescence, dates back to the 16th century[1]. During the following centuries, investigations of the phenomenon have been made by several scientists for example Newton, Brewster and Stokes. Today, fluorescence has found an immense variety of applications in various fields of science.

Fluorescence spectroscopy is nowadays a widely used technique which is characterised by its extraordinary high sensitivity, enabling the detection of a single molecule. In the exploration of photophysical and photochemical processes of electronically excited molecules, the utilising of fluorescence spectroscopy has become indispensible.

Employment of fluorescence techniques in chemistry and biology provides information about e.g. structure, dynamics, and interactions between molecules. Moreover, fluorescence techniques are also widely used in a combination with other imaging techniques, e.g. in the visualisation of processes in cells and biological tissues. Time-resolved fluorescence has proved to be explicitly suitable to monitor excited state dynamics of molecules, which is appropriate for the study of electronic energy transport between chromophoric molecules. Electronic resonance energy transfer (RET) can be used to obtain information concerning structure and conformational dynamics in bio-macromolecules, such as DNA and proteins. In 1948, Theodor Förster derived a theory of RET, starting from quantum mechanics[2]. As an important result, Förster’s theory relates, the strongly distance dependent, rate of energy transfer to experimental observables. By measuring the rate of RET between two chromophoric molecules information about their mutual distance can be obtained. RET complements techniques more commonly used to gain structural information such as X-ray and multidimensional NMR.

This thesis aims at investigating the influence of the reorientational dynamics of interacting fluorescent probes under different circumstances of electronic energy transport. The thesis presents an extended Förster theory (EFT) of electronic energy transfer that provides a more detailed molecular description
as well as a quantitative analysis of time-resolved fluorescence lifetime and depolarisation experiments. Furthermore, the EFT accounts for reorienting fluorophores in the case of partial donor-donor energy migration (PDDEM).

In Förster theory of dipole-dipole coupling between interacting chromophores, their joint distance ($R$) together with the orientation factor ($\kappa^2$) dictate the rate of energy transport. Unfortunately, often the method cannot give an atomic resolution of distances due to the uncertainties in the chromophore motions. However, based upon recently developed theories of electronic energy transport, a solution to the in literature extensively discussed “$\kappa^2$-problem” for two specific cases, DAET and PDDEM is presented and thereby overcoming the limitations of the hitherto used RET methods.

2. Fundamental concepts

This section introduces the physical origin of fluorescence, as well as the basic concepts of fluorescence spectroscopy. The overview given is valid for fluorescence in the condensed phase, which is relevant for most of the studied systems in chemistry and biosciences.

2.1 Fluorescence

The phenomenon of fluorescence in optical spectroscopy is defined by spontaneous emission of a photon from an electronically excited state, mediated through a spin-allowed transition. A spin-forbidden emission is referred to as phosphorescence[3]. These processes constitute a subclass of luminescence, which is a general concept for the emission of photons from electronically excited states. There are several possible pathways of relaxation of an electronically excited state, of which most are non-radiative. Emission of light from a molecule in a solvent under normal conditions will predominately be from the lowest electronic singlet excited state in which the molecule has
relaxed into the ground state of its vibrational modes. The process of vibrational relaxation (VR) is rapid as compared to the lifetime of the electronically excited state and occurs typically on the ps timescale. The radiative transitions from the first excited singlet state usually occur on the ns timescale.

According to the Boltzmann distribution, most molecules occupy the electronic ground state at equilibrium and room temperature. The common nomenclature denotes the singlet ground state as: \((S_0)\), the excited singlet states: \((S_1, S_2,\ldots)\), triplet states: \((T_1, T_2,\ldots)\) and vibrational states: \((v_1, v_2,\ldots)\). The ground state \(S_0\) can also be reached by an internal conversion (IC) process, whereby a non-radiate conversion occurs from an electronically excited state in a vibrational energy level to a lower matching electronic state, but with balancing higher vibrational level schematically written as: \((S_n, v_k) \rightarrow (S_{n-1}, v_m)\) and then followed by vibrational relaxation. Another pathway of relaxation is a non-radiative transition from the singlet state \(S_i\) to the triplet state \(T_i\) (vibrational states omitted) termed: intersystem crossing (ISC), which is followed by phosphorescence. The various relaxation pathways are conveniently summarised in a Jablonski diagram[3].

The next section demonstrates how the polarisation of fluorescent light can be used to gain structural and dynamic molecular information.

2.2 Fluorescence depolarisation

Fluorescence depolarisation experiments provide useful molecular information e.g. about the reorientation and the order of chromophoric molecules in lipid bilayers, as well as structural insights concerning proteins. In the following, only macroscopically isotropic systems are considered.

The selective excitation of molecules with respect to their orientation is most often referred to as photoselection. A fluorophore absorbs light with its highest probability if the electronic transition dipole is oriented parallel to the electric field vector of the impinging light. The probability of excitation is proportional to \((\vec{E} \cdot \vec{\mu})^2\), where \(\vec{E}\) is the electric field vector and \(\vec{\mu}\) is the absorption electric
dipole moment vector of the transition. By using linearly polarised light for the excitation, the created orientational distribution of electronically excited molecules becomes uniaxially oriented about the $\vec{E}$-field, i.e. the excited molecules have a preferred orientation in space. At the time of emission, molecular reorientations may have occurred, which have caused a depolarisation of the emitted fluorescence relative to the orientation of the incident light. Experimentally, the fluorescence anisotropy is introduced in order to determine depolarisation. The steady-state fluorescence anisotropy can be calculated from the following expression[3]:

$$ r_{ss}(\lambda) = \frac{F_{VV}(\lambda) - G(\lambda)F_{VH}(\lambda)}{F_{VV}(\lambda) + 2G(\lambda)F_{VH}(\lambda)} $$  \hspace{1cm} (2.1) 

Here, $F_{VV}(\lambda)$ and $F_{VH}(\lambda)$ denote the fluorescence spectra. The first subscript is referring to vertically polarised light (V) used for excitation, while the second subscript refers to vertical and horizontal settings for the emission polariser, respectively. $G$ is the instrumental correction factor[3]:

$$ G(\lambda) = \frac{F_{VV}(\lambda)}{F_{VH}(\lambda)} $$  \hspace{1cm} (2.2) 

Accordingly, in a similar fashion the time-dependent anisotropy is defined for fixed values on the wavelength of excitation and emission:

$$ r_{\exp}(t) = \frac{F_{VV}(t) - gF_{VH}(t)}{F_{VV}(t) + 2gF_{VH}(t)} = \frac{d_{\exp}(t)}{s_{\exp}(t)} $$  \hspace{1cm} (2.3) 

Here, $d_{\exp}(t)$ denotes the difference curve and $s_{\exp}(t)$ the sum curve. The instrumental correction factor, $g$ is obtained from an expression similar to the steady-state correction factor or by using the steady-state anisotropy[3]. The $s_{\exp}(t)$ curve only contains information about the photophysics relaxation, and moreover is directly proportional to the total intensity emitted from a sample. Hence $s_{\exp}(t)$ is independent of the rotational reorientation of the molecules. The photophysics relaxation is also available from a single experiment in which the excitation polariser is set to vertical while the emission polariser is
set at 54.7° relative to the vertical axis, which is often referred to as magic angle (MA) settings of the polarisers. The difference curve $d_{\text{exp}}(t)$ depends on both the fluorescence relaxation and the anisotropy.

In summary, the fluorescence depolarisation can be expediently quantified by the fluorescence anisotropy $r(t)$. The molecular origin to the time-dependent depolarisation is often caused by rotational motions of the fluorophores. The energy migration process, however, also influences the depolarisation, as will be apparent from the following chapters.

3. Theoretical framework

Resonance energy transfer (RET), or Förster resonance energy transfer (FRET), means radiationless transfer of electronic energy from a donor to an acceptor molecule. The physical origin most often described by a long range dipole-dipole interaction. It is a widely used phenomenon in studies of biomolecules. From such experiments information about distances typically in the range of 20-90 Å can be obtained. In 1948, Theodor Förster presented the first quantum mechanical description of electronic energy transfer[2]. This theory constitutes the classical Förster theory, which is cited in myriads of scientific papers. Approximately two decades later, an important experimental support of the Förster theory was published by Stryer and Haugland [4]. They suggested that RET could be used as a “chemical ruler” in biosciences. The electronic energy transport processes treated in this thesis can be categorised as an extended Förster theory of; (1) irreversible energy transfer i.e. an unidirectional transfer of electronic energy from a donor to an acceptor molecule, and (2) partial reversible energy migration, whereby electronic energy migrates between two fluorophores with different probabilities. Reversible energy migration was considered only to a minor extent.
3.1 Donor-acceptor energy transfer (DAET)

Donor-acceptor energy transfer (DAET) between two interacting fluorophores can be used as an example of RET and pictured according to the following kinetic scheme:

$$D(S_1) + A(S_0) \rightarrow D(S_0) + A(S_1)$$

A donor in its first excited electronic state, $D(S_1)$, interacts in a non-radiative process with an acceptor in its electronic ground state, $A(S_0)$, whereby the donor relaxes to its ground state while the acceptor becomes excited. The radiationless transfer of electronic energy occurs provided that there is a spectral overlap between the donor’s emission spectrum and the acceptor’s absorption spectrum. The rate of energy transfer ($\omega_{DA}$) is related to the distance between the interacting fluorophores ($R$) according to [2]:

$$\omega_{DA} = \frac{3}{2 \tau_0} \left( \frac{R_0}{R} \right)^6$$

(3.1)

Here, $\tau_0$ and $R_0$ stand for the radiative lifetime and the Förster radius, respectively. The rate, $\omega_{DA}$, is evidently strongly distance dependent. Moreover, the equation includes a quantity referred to as the Förster radius, $R_0$. The latter is given by:

$$R_0^6 = \frac{9000(\ln10)\kappa^2 J}{128\pi^3 n^4 N_A}$$

(3.2)

In Eq. 3.2, $n$ and $N_A$, is the refractive index[5] and Avogadro’s constant respectively. Note that the rate of energy transfer is invariant to the quantum yield of fluorescence. The overlap integral can be calculated from:

$$J = \int F_D(\lambda) \varepsilon_A(\lambda) \lambda^4 d\lambda$$

(3.3)
Furthermore, the angular part of the transfer rate is given by the orientation factor:

$$\kappa^2 = (\cos \delta - 3 \cos \beta_1 \cos \beta_2)^2$$  \hfill (3.4)

The angle between the transition dipole moments is denoted \(\delta\), whereas \(\beta_1\) and \(\beta_2\) are the angles to a common vector \((R)\) that interconnects the two interacting transition dipole moments (cf. Fig. 1, Paper I).

The orientation factor is of utmost importance since it influences the transfer rate. The uncertainties due to \(\kappa^2(t)\) have hitherto undisputedly been a setback for the strive of accuracy when applying energy transfer techniques. In the preceding decades several attempts have been made to account for the uncertainties in the orientation factor\([6,7]\), e.g. by making use of fluorescence depolarisation measurements to obtain limiting values of \(<\kappa^2>\). The present thesis overcomes the frequently appearing “\(\kappa^2\)-problem”.

3.2 Reversible and partially reversible energy migration

Among chemically identical fluorescent molecules, exhibiting the same single exponential photophysics, the rate of energy transport is reversible, meaning that the electronic energy can migrate with equal rates within a pair of interacting molecules. Hence, the energy migration is a better suited concept than transfer\([8]\) to distinguish from the DAET process. Chemically identical is a concept used to refer to fluorophores that exhibit identical spectral overlap of the absorption and emission spectrum. Conventional studies of proteins, using DAET, involve specific labelling of the protein molecules with one donor and one acceptor group. In practice this is a critical step and most often difficult to perform. Having that in mind, the idea of using DDEM for distance measurements appears attractive because the need for site specificity in labelling is circumvented.

In the case of DDEM, it is important that the donors exhibit identical single exponential photophysics, which implies that the observed fluorescence relaxation is invariant to the rate of energy migration. Information concerning
the energy migration process can however, be accessed from fluorescence depolarisation experiments. It is worth to note that DDEM was one of the first observations of electronic energy transport in solution[9]. One limitation in the application of DDEM is the need of using fluorescent probes, whose light spectroscopic properties are insensitive to their microenvironment. Most fluorophores do not meet this criterion and in practice, only a limited number of probes are available. Nevertheless, it is still possible to gain information from non-ideal DD-pairs, although the energy migration transport is only partially reversible.

The special case of PDDEM considered here refers to energy migration between chemically identical, but photophysically non-identical fluorophores. That is, the donors are of the same kind of chemical species, while their fluorescence relaxation rates are different or show multi-exponential fluorescence relaxation patterns. Variations in fluorescence relaxation can be expected e.g. due to different labelling sites. For the fully asymmetric case, $\omega_{a\beta} \neq \omega_{\beta a}$ and $\tau_a \neq \tau_b$, an analytical model of PDDEM has previously been derived[10]. The derivation is based on the classical Förster theory. For the special case of equal energy migration rates, extensive studies have been performed using an analytical form of PDDEM and the extended Förster theory of PDDEM (cf. Papers II, III). Fig. 3.1 illustrates this particular case of PDDEM.

Contrary to DDEM, not only the fluorescence anisotropy, but also the fluorescence relaxation of the coupled system, contains information regarding

![Fig. 3.1](image_url)
the energy transport. Hence, information regarding distances can be determined from both fluorescence relaxation and depolarisation experiments.

3.3 Extended Förster theory (EFT)

EFT allows theoretical treatment of the energy transfer/migration within reorienting and weakly dipole-dipole interacting pairs of fluorophores. The fluctuations in $\kappa^2(t)$ and consequently the $\omega(t)$ function, need to be considered in order to correctly account the dipole–dipole interaction on the timescale of interaction.

In the beginning of the 1990:ies, Westlund and Wennerström [11] introduced a Liouville formalism for treating energy transfer processes within a common framework of relaxation processes. Later, algorithms were introduced that enabled solutions for describing energy migration within a pair of reorienting fluorophores, using the stochastic Liouville equation (SLE)[12]. The stochastic motions of the interacting fluorophores can be accounted for by using Brownian dynamics (BD) simulations. In 1996, Johansson et al.[13] introduced the extended Förster theory, presenting a stochastic master equation (SME) of energy migration between two donors, which was derived from the SLE. EFT accounts for energy migration within pairs of fluorophores that undergo reorientational motion on the timescale of interaction by modelling the stochastic reorientation of the fluorophores transition dipole moments. This is further detailed in the next section.

3.4 Computational methods

In order to describe the stochastic behaviour of $\kappa^2(t)$, as well as the excitation probabilities that appear in the EFT, the reorienting motions of the fluorophores need to be modelled. Throughout this thesis, BD simulations have been used to mimic the molecular reorientations. The orientation of the electronic excitation and emission transition dipoles of the fluorophores are primarily influencing $\kappa^2(t)$. A model, or potential was chosen to restrict the simulated stochastic motions.
3.4.1 Ordering potentials

Different modelling potentials can be used to mimic restricted molecular motions in microscopically isotropic and anisotropic systems. Frequently, the rather simple yet effective cone potential is applied. The cone model allows restricted diffusion of a unit vector within the boundaries of a cone defined by the semi-cone angle ($\beta_C$). The simulations generate a trajectory of the time-evolution of a stochastically reorienting vector. The simulation algorithm used here falls back on that presented by Fedchenia et al.[14]. Represented by a unit vector, the coordinates of the transition dipole moment evolve according to:

\begin{align*}
x_{i+1} &= x_i + \zeta_x \sqrt{D_c h} \\
y_{i+1} &= y_i + \zeta_y \sqrt{D_c h} \\
z_{i+1} &= z_i + \zeta_z \sqrt{D_c h}
\end{align*}

In Eq. 3.5, the subscript $i$ represents an index of time, $h$ is the time-step and $D_c$ denotes the diffusion constant whereas $\zeta_{x,y,z}$ are Gaussian random numbers with standard deviation of one and zero mean value. In every time-step the coordinates are projected down to a unit sphere in order to preserve the reorientation. The above equation exemplifies that generation of random numbers is an essential part of generating BD trajectories. The routines used for that was originally written by Marsaglia and Zaman [15]. Given the width of the cone, i.e. $\beta_C$, Lipari and Szabo [16] have derived an analytical expression of the integrated rotational correlation time. In simulations, the calculated correlation times can thus be compared with the predicted ones as a control.

The fluorophores within a macromolecule are considered to reorient independently. Trajectories are simulated for each molecule in a donor-donor or donor-acceptor pair. If the fluorophores are covalently attached to a macromolecule at different locations, the use of different potentials e.g. different diffusion constants and cone angles might be needed. Examples of highly flexible models for the reorientational motions can be achieved by combining two independent diffusion processes[17].
3.4.2 Data analysis

To address questions that concern the dynamics of excited state relaxation, time-resolved fluorescence techniques are most useful. In this thesis, time-resolved fluorescence data are presented using the time-correlated single photon counting (TCSPC) approach. TCSPC experimental data inherently obeys Poisson statistics[18]. The well-defined statistics of the data accumulation is most valuable for a quantitative evaluation of the experiments with respect to different models. To correctly account for the characteristic of the experimental TCSPC setup, the model \( m \) is compared to the experimental data after a convolution with the instrumental response function (IRF).

\[
F_m(t) = \int_0^t m(t - t') IRF(t' + \eta) dt' = m(t) \otimes IRF(t + \eta) \quad (3.6)
\]

Here, \( F_m \) is the calculated decay and \( \sigma, t, \) and \( t' \) denote time with \( \eta \) as a variable time shift parameter. The convolution operator is represented by \( \otimes \).

The evaluation of the best fit of the model to experimental data is usually performed by using a statistical criterion, such as the reduced- \( \chi^2 \) test:

\[
\chi^2_i = \sum_{i=n_1}^{n_2} \left[ \frac{F_{\text{exp}}(t_i) - F_m(t_i)}{\sigma^2(t_i)} \right]^2 \int_0^1 (p_2 - p_1 - 1) \quad (3.7)
\]

Here \( \chi^2_i \) denotes the goodness of fit function, \( F_{\text{exp}} \) stands for the experimental data, \( \sigma \) is the standard deviation of point (channel) \( i \), \( p \) denotes the number of fitting parameters and \( n_1 \) to \( n_2 \) defines the interval of the fit. Often a graph of the weighted residuals and the autocorrelation function are helpful in the analysis.
4. Results and discussion

In this section results from the included Papers I-III are summarised. The focus will be on the extended Förster theory and its relation to experimental data as well as to previous work on DDEM within donor-donor pairs. The novelty concerns the theoretical treatment and methodology of EFT in the context of DAET and PDDEM.

When analysing energy transfer/migration experiments, the obtained rate of energy transfer needs to include the correct influence of the orientation factor in order to determine the distance between the chromophores. The recurrent “κ²-problem” has been discussed in literature[19]. Additionally, something less highlighted, but of importance is the correlation between the energy transfer process and the reorientational dynamics. The extended Förster theory addresses these questions, which are relevant for PDDEM as well as DAET experiments.

4.1 Extended Förster theory of PDDEM

Describing physical processes as stochastical processes is not a trivial task. Fortunately, irreversible processes in a quantum mechanical subsystem (S) embedded in either a classical or quantum mechanical environment (L) can thus be treated advantageously using the stochastic Liouville equation (SLE). SLE offers the possibility to let classical degrees of freedom influence quantum mechanical degrees of freedom, but not the other way around. This implies an introduction of the asymmetry needed to describe relaxation. Using SLE, the information of the density operator is available from a full quantum mechanical treatment. The wavefunctions are lost but for the expectation values corresponding to transition probabilities, only density matrix elements are needed. The gain with the superoperator formalism is the elegant and concise algebra that it introduces.

The starting point is the SLE given in the Langevin form[20]:

18
\[
\frac{\partial}{\partial t} \chi(t) = -i\left[L^0_S + L_{SL}(\Xi(t))\right]\chi(t)
\]

(4.1)

The Langevin approach is appropriate for the description of relaxation in the time domain. The superoperators \(L^0_S\) and \(L_{SL}[\Xi(t)]\) refers to the isolated subsystem and the coupling between the subsystem and the environment respectively. Here, \(\Xi(t)\) denotes stochastic variables. The Liouville superoperators are defined as a commutator of the Hamiltonian of the system treated. The statistical density operators \(\chi(t)\) are a direct product of the eigenfunctions of the Hilbert space of two 2-level systems.

In the work presented in Paper II, the stochastic Liouville equation accounts for electronic energy migration in a subsystem, assuming two electronic 2-level systems. The Liouville super operator matrix is a 4 x 4 matrix. There are four relevant electronic density matrix elements of \(\chi(t)\):

\[
\chi_1 = |0\rangle\langle 0|, \quad \chi_2 = |1\rangle\langle 1|, \quad \chi_3 = |0\rangle\langle 1|, \quad \chi_4 = |1\rangle\langle 0|
\]

(4.2)

Using similar notation as in Paper II, the stochastic Liouville matrix of the photophysics of donor \(\alpha\) and \(\beta\) is given by:

\[
iL(t) = i\left[L^0_{\alpha}(t) + L_{\alpha\beta}(\Omega(t))\right] =
\]

\[
\begin{bmatrix}
1/\tau_\alpha & 0 & iH_{\alpha\alpha}(\Omega(t)) & -iH_{\alpha\beta}(\Omega(t)) \\
0 & 1/\tau_\beta & -iH_{\beta\alpha}(\Omega(t)) & iH_{\beta\beta}(\Omega(t)) \\
iH_{\alpha\alpha}(\Omega(t)) & -iH_{\alpha\beta}(\Omega(t)) & 2/T_2 + i\Delta\nu & 0 \\
iH_{\beta\alpha}(\Omega(t)) & iH_{\beta\beta}(\Omega(t)) & 0 & 2/T_2 - i\Delta\nu
\end{bmatrix}
\]

(4.3)

Here, \(T_{2\alpha}=T_{2\beta}=T_2\) is the relaxation time, responsible for the spectral bandwidth and \(\Delta\nu\) stands for the difference in frequency between the absorption and fluorescence spectra of the donor molecules i.e. the Stokes’ shift. The
The electronic transition Hamiltonian operator for dipole-dipole interactions is given by:

\[
H_{dd}(\Omega(t)) = f_R \frac{\kappa[\Omega(t)]}{\sqrt{2}} \left( |00\rangle \langle 00| + |01\rangle \langle 01| \right)
\]  

(4.4)

\[
f_R = \frac{\mu^* \mu}{4\pi\varepsilon_0 \varepsilon R^3}
\]

The angular part of the dipole-dipole coupling is given by \(\kappa(t)\). From a perturbation treatment of the SLE (Eqs. 4.1-4.3), a master equation can be obtained:

\[
\begin{bmatrix}
\dot{\chi}_1(t) \\
\dot{\chi}_2(t)
\end{bmatrix} =
\begin{bmatrix}
-1/\tau_a - \omega(t) & \omega(t) \\
\omega(t) & -1/\tau_\beta - \omega(t)
\end{bmatrix}
\begin{bmatrix}
\chi_1(t) \\
\chi_2(t)
\end{bmatrix}
\]

(4.5)

In the derivation of Eq. 4.5, the Markov approximation was utilised, \(\xi_i(s) \approx \xi_i(t)\), and a perturbation assumption due to different timescales denoted “coarse graining”. The memory effect in the dipole-dipole coupling can be neglected because \(T_2 << \tau_\alpha, \tau_\beta\) [21]. The transfer rate in Eq. 4.5 is given by:

\[
\omega(t) = f_R^2 \kappa^2(t) \frac{T_2}{2 + (\Delta \nu T_2)^2}
\]

(4.6)

From identification with Förster’s theoretical expression [2,3] (cf. Eq. 3.1) for the rate of energy migration one obtains that the Förster radius can be rewritten as [13]:

\[
R_0 = \frac{2}{\sqrt{3}} f_R^2 R^4 \left( \frac{T_2}{2 + (\Delta \nu T_2)^2} \right)
\]

(4.7)

Eq. 4.5 cannot however be solved using perturbation theory/Redfield theory [22] when the rate of fluorescence and the transfer rate are on same order of magnitude, which Paper II exemplifies. Moreover, Eq. 4.5 can neither be reduced to one-dimension as the case of DDEM [13]. However, there have been attempts to analytically solve the SME by using cumulant series expansions [23]. These expansions are however often truncated after the second term. Notably, the first cumulant is identical with the classical Förster
expression. In general the cumulant expansion of first order overestimates the rate of migration. To compensate, the second cumulant expansion is supposed to provide an impeding correction of the rate. Unfortunately the second cumulant expansion diverge when $\kappa(t)$ and $\omega(t)$ fluctuates in similar time regimes[13]. For that reason no attempts of cumulant approximations have been made for EFT of PDDEM.

The procedure for numerically solving Eq. 4.5 is simplified by choosing another set of basis operators:

$$
\begin{bmatrix}
\xi_1(t) \\
\xi_2(t)
\end{bmatrix} = e^{\xi(t)} \begin{bmatrix}
\chi_1(t) \\
\chi_2(t)
\end{bmatrix}
$$

(4.8)

Then, the integrated form of Eq. 4.5 can be written:

$$
\begin{bmatrix}
\xi_1(t) \\
\xi_2(t)
\end{bmatrix} = \exp\left\{\int_0^t 2\omega(s)P(s)ds\right\} \begin{bmatrix}
\xi_1(0) \\
\xi_2(0)
\end{bmatrix}
$$

(4.9)

Where $P(s)$ is a two-dimensional matrix (cf. Eq. 4.12).

Methods using BD are needed to generate the stochastic process of $\kappa^2(t)$ and indirectly $\omega(t)$. The superoperator $\xi(t)$ will then be averaged over a large number of BD trajectories.

It was noted that the numerical calculations of the stochastic integral in the exponent of Eq. 4.9 can be simplified for small time steps ($\Delta t$) and be rewritten according to:

$$
\begin{bmatrix}
\xi_1(t_{n+1}) \\
\xi_2(t_{n+1})
\end{bmatrix} = \left\{1 - \exp\{-2\omega(t_n)\Delta t\}\right\}P(t_n) \begin{bmatrix}
\xi_1(t_n) \\
\xi_2(t_n)
\end{bmatrix}
$$

(4.10)

Using the fact that the matrix exponent can be expanded into an infinite series that algebraically can be formulated as:

$$
\exp\{2\omega(t_n)P(t_n)\Delta t\} = 1 - \left\{\exp\{-2\omega(t_n)\Delta t\} - 1\right\}P(t_n)
$$

(4.11)

Here, $P(t_n)$ is given by:
\[
\mathbf{P}(t_a) = \begin{bmatrix}
-1/2 \\
(1/2)\exp[(\tau_0^{-1} - \tau_a^{-1})t_a]
\end{bmatrix}
\]

where \( \mathbf{I} \) denotes the identity matrix. Since the infinite series is kept there is no loss of accuracy in this step.

With the excitation probabilities at hand, the task is then to derive \( F_{VV} \) and \( F_{VH} \), the observables for TCSPC. Assuming equal probabilities of absorption for both donors, the observed theoretical fluorescence decay \( s(t) \) of the coupled \( \text{D}_\alpha\text{D}_\beta \)-pair is given by:

\[
s(t) = \frac{1}{2} < \chi^0_\alpha(t) + \chi^0_\beta(t) + \chi^1_\alpha(t) + \chi^1_\beta(t) >
\]

In this notation (cf. Paper II), \( \chi^0_\alpha(t) \) and \( \chi^1_\alpha(t) \) can be defined using the conditional probabilities \( \chi^0_\alpha(t) = \chi_\alpha(t|\chi_\alpha(0)) \), \( \chi^1_\alpha(t) = \chi_\alpha(t|\chi_{\alpha'}(0)) \).

I, J \( \in \alpha, \beta \). The observed fluorescence decay is not invariant to the energy migration process, as is the case in DDEM. Using the relation needed for describing \( F_{VV} \) and \( F_{VH} \) one finally obtains:

\[
F_{VV}(t) = C \sum_{I=\alpha,\beta} \langle 1 + 2r_\alpha T_{\alpha \beta}(t) \rangle \chi^0_\alpha(t) + \sum_{I=\alpha,\beta} \langle 1 + 2r_\beta T_{\alpha \beta}(t) \rangle \chi^1_\alpha(t)
\]

\[
F_{VH}(t) = C \sum_{I=\alpha,\beta} \langle 1 - r_\alpha T_{\alpha \beta}(t) \rangle \chi^0_\alpha(t) + \sum_{I=\alpha,\beta} \langle 1 - r_\beta T_{\alpha \beta}(t) \rangle \chi^1_\alpha(t)
\]

(4.14)

Where \( \rho_\alpha(t) = P_2[\hat{\mu}_\alpha(0) \cdot \hat{\mu}_\alpha(t)] \) \( \text{I}, \text{J} \in \alpha, \beta \)

(4.15)

In Eq. 4.15, \( P_2 \) is the second-rank Legendre polynomial \( P_2 = (3x^2 - 1)/2 \) and e.g. \( \hat{\mu}_\alpha \) stands for a unit vector of the molecule \( \alpha \), directed along the electronic
transition dipole moment vector. The applied Liouville formalism works well in the working range of the dipole-dipole approximation. From calculations expressing quantum electrodynamics, it has been shown that radiationless and radiative energy transfer are the short- and long-range asymptotes of a unified mechanism[24]. To correctly formulate electronic energy transport occurring at shorter distances than approximately 10 Å, quantum electrodynamics is required. However, distances between chromophores i.e. the length scale of interest, are exclusively well within the approximation.

4.2 Analyses of DA experiments

The fluorescence decays observed in presence of electronic energy transfer depend on the coupling strength, the reorientation of the interacting groups as well as their orienting configuration, accounted for by the Kubo number (cf. Eq. 4.19). Any attempt to extract all parameters directly from a donor-acceptor fluorescence relaxation experiment truly needs a strenuous effort. Fortunately, fluorescence depolarisation studies of the D and A groups in the absence of coupling, provide knowledge regarding the reorientation correlation times and the orientational restrictions of the fluorophores, the latter information is described in terms of order parameters. According to Paper I, in presence of an acceptor the sum curve for the donor emission can be written:

\[ s(t) = F_D(t)\langle \chi(t) \rangle \]  \hspace{1cm} (4.16)

The difference curve for the donor (D) is given by:

\[ d(t) = F_D(t)\langle \chi(t)P_2[\hat{\mu}_D^R(0) \cdot \hat{\mu}_D^R(t)] \rangle \] \hspace{1cm} (4.17)

In Eqs. 4.16-4.17, \( F_D(t) \) denotes the fluorescence relaxation of the donor in the absence of coupling. The probability \( \chi(t) \), means that an initially excited donor is still excited after a time \( t \), hereafter referred to as excitation probability (cf. Paper I). Note that the observables derived (or quantities constructed from observables) refer only to the donor molecule.
From experiments, additional information about the configuration could be obtained from the acceptor. Both the acceptor fluorescence relaxation and anisotropy will be influenced by energy transfer. In principle, DAET experiments provide four different observables that report on the same rate of energy transfer, which potentially opens the possibility to reveal the configuration of the system together with the inter-fluorophore distance.

The dynamic average rate of energy transfer $\langle \omega \rangle$, in a DAET experiment, is only valid in the initial slope of the fluorescence relaxation decay of the donor. It was demonstrated that an analytical fit of only the initial part of the decay could provide a slightly better fit than analysing the whole decay, when reanalysing synthetic data using FT.

4.3 EFT analysis of TCSPC data

Starting with a coupled system (e.g. a macromolecule labelled with two fluorophores), the parameters to extract in an analysis of data from a TCSPC experiment to describe the energy transport process are the coupling strength ($\Lambda$) and the three configuration angles ($\beta_1, \beta_2, \alpha$). To search for the relevant information a “screening method” has been suggested, which appear in Paper I and II. By this method, a large number of configurations (typically in steps of 1-5°) were investigated for a range of $\Lambda$-values. For each $\Lambda$-value the $\chi^2$-values were calculated. Apart from brute force, the method imposes restrictions on the allowed configuration angles. These restrictions differ depending on type of energy transport. In DAET, information from the initial slope of the fluorescent relaxation decay can be used to estimate the first cumulant i.e. the exponent $\Lambda\langle \kappa^2 \rangle$. For a given lambda, the configuration space can then be reduced to all configurations fulfilling the first cumulant. Additionally, the maximum and minimum values of $\langle \kappa^2 \rangle$ can be calculated from Eq. A2 in Paper I, with knowledge of the order parameters. In addition, Eq. 4.18 imposes restrictions to the configuration:
\[
\cos \delta = \cos \alpha_{DA} \sin \beta_D \sin \beta_A + \cos \beta_D \cos \beta_A \\
|\beta_D - \beta_A| \leq \delta \\
|\beta_D + \beta_A| \geq \delta
\] (4.18)

By combining these approaches the configuration space can be reduced significantly. In Fig. 4.1, the lowest $\chi^2$-value have been plotted against $\Lambda$, exemplifying what knowledge of the value of the $\delta$-angle provides to the analysis. It is evident that there is a range of solutions to the EFT that fits well with the synthetic data. This was however, not further analysed since distance information is usually the most interesting parameter. The range in $\Lambda$ can be translated into distances. By using $R_0 = 50 \, \text{Å}$, the ranges correspond to 5.6 Å and 7.0 Å for panel A and B respectively. The minimum in Fig 4.1 panel A could also be described as more distinct. One should further notice that the predicted interval of $\Lambda$ by other methods are much wider[7].

Often, the Kubo number has been used to characterise the dynamic contribution to $\omega(t)$[20].

\[
\vartheta = \Lambda \tau_\kappa \left\{ \left( \kappa^4 \right) - \left( \kappa^2 \right)^2 \right\} = \Lambda \tau_\kappa \Delta \kappa^4
\] (4.19)

In the equation above, $\Delta \kappa^4$ denotes the variance, $\tau_\kappa$ is the integral correlation time of the correlation function $\langle \kappa^4(0) \kappa^4(t) \rangle$ and its value is normally calculated numerically from the simulations.

In Fig. 4.1, for the system corresponding to a higher Kubo number ($\vartheta = 4.2$) the correct solution is found while for the second case with $\vartheta = 1.5$ two possible solutions are found. Even though both configurations have matching coupling strength, the values of the $\kappa^2$-correlation time ($\tau_\kappa$) and the variance are different. As the Kubo number decreases, the information content effectively decreases for fluorescence relaxation decays obtained for the two coupling chromophores. For $\vartheta \ll 1$ the first cumulant is a valid approximation.
When independent information can be provided regarding the configuration it is possible to considerably reduce the configuration space. In DAET there are multiple ways for this, an implication of such a restriction is demonstrated above. Similar screening methods have been applied on protein system exhibiting DDEM with success[17]. The photophysics and depolarisation data screening of PDDEM experiments are displayed in Fig 4.2. The data were analysed without prior knowledge of the true parameter values, as well as assuming a known value on the angle, δ ± 5°. Although there is no theoretical expression for how to obtain the δ-angle in PDDEM there are indications on how to attain such information from numerical analysis, described in Paper II.
Fig. 4.2 Above, is the best fit value $\chi^2_{\text{tot}}$ averaged over both $s(t)$ and $d(t)$ as a function of $A$ when the whole configuration space was covered. All permitted values of the $\delta$-angle were allowed for the curve marked with the squares, (■) whereas the only the correct value of $\delta \pm 5^\circ$ was accepted for the curve indicated with diamonds (♦). Here, $d(t)$ is responsible for most of the deviation. For the system examined the correct values of the parameters were: $\alpha = 11^\circ$, $\beta_1 = 40^\circ$, $\beta_2 = 40^\circ$, $\delta = 7.06^\circ$ and $A = 0.50$. Moreover the dynamic value of $<\kappa^2>$ = 0.669 and $\vartheta = 0.7$. The orienting potential is defined by the cone angle $\beta_c = 25^\circ$. A grid of 1° was used in scanning the configuration space (cf. Fig. 5 in Paper II).

The procedure here described for analysing the TCSPC data has the advantage over traditional least-square-fitting algorithms by giving information about the uncertainty in the values for the obtained distances. Unfortunately, they have the passing drawback of being very time-consuming. From Fig. 4.1, the difficulties in applying a normal minimising routine are apparent when several minima’s or a range of equally low $\chi^2$ exists. It should be noted that in the present studies the statistical noise in TCSPC, described by Poisson statistics, was considered as the source of error. Other sources of errors might appear when analysing real experimental data.

In both minimisation routines and screening methods a convolution with the IRF is needed before the model curve can correctly be compared with the
experimental data. However, this comparison will not necessarily be correctly evaluated unless an amplitude factor is used to compensate for different acquisition times and intensities. Therefore, in the optimisation routines such amplitude factor is used. However, this parameter is for many cases redundant. It has been noted that by using a multiplying factor, consisting of the ratio between the integrated model curve and the integrated experimental curve, instead of an amplitude factor could be equally descriptive in the analysis. Hence, one fitting parameter could possibly be reduced in the analysis.

Gradient or steepest descent analysis, like nonlinear least-squares fitting[25] e.g. the Levenberg-Marquardt algorithm, in conjunction with semi brute force methods such as the screening method described in Paper I-II, are the minimisation routines used in this thesis. For complex or noisy data, evolutionary stochastic methods like genetic algorithms[26] or maximum entropy modelling can be applied[27].

4.3.1 Generating and re-analysing synthetic TCSPC data

For generating synthetic TCSPC data, the method proposed by Chowdhury et al.[28] has been used. The distinctive Poissonian statistics of the method enables a realistic modelling of TCSPC. Thus, synthetic experimental data can be re-analysed to investigate e.g. how well model parameters can be recovered within a desired statistical accuracy. Problems to recover the correct parameters in the analysis of the synthetic data, would strongly hint that certain difficulties would be expected for the corresponding analysis of real experiments. The model optimisation is carried out the same way as for real experiments i.e. by a minimization of $\chi^2$ using appropriate search algorithms.
4.4 EFT and fluorescence spectroscopic properties

In the beginning of this chapter, the EFT of PDDEM was outlined resulting in the derived observables $F_{VV}(t)$ and $F_{VH}(t)$. There are however other measurable quantities associated with electronic energy transport. For the study presented in Paper III, the aim was to examine the influence of electronic energy transport and reorientational dynamics within pairs of chemically and photophysically identical chromophores. However, the photophysics was assumed not to be mono-exponential. For convenience, the fluorescence relaxation of each donor ($\alpha$ or $\beta$) were modelled by equal bi-exponential fluorescence decays in the absence of Förster coupling. In the presence of energy transport, DDEM occurs between the species of equal lifetime ($i.e.$ $\tau_{\alpha i} = \tau_{\beta i}$), whereas PDDEM occurs between the species of different lifetimes. The intension is to illuminate the influence of this complexity on various commonly studied fluorescence spectroscopic properties.

Normally, it would be assumed that the relative quantum yield is unity for DDEM. However, this is not true as is shown by the calculated data. The relative fluorescence quantum yields have been calculated for different configurations and strengths of coupling ($cf.$ Table 4.1-4.2). In general, the obtained values from EFT and the FT-approximation are rather similar. Since the typical errors in quantum yield determinations are 5-10 $\%$, the value obtained by the FT approximation is usually experimentally indistinguishable from the EFT result.

The steady-state fluorescence anisotropies obtained for different configurations and strengths of coupling were calculated. Some of the attained results can be found in Table 4.2. The true values as obtained from the EFT are compared with the values predicted by using the common approach of the Förster theory. The steady-state values predicted by the EFT were often found to be somewhat higher than those acquired within the FT for rotational correlation times, $\phi_c = A^{-1}\langle k^2 \rangle^{-1}$. For the case of an almost perpendicular configuration ($cf.$ $\Omega = IV$ in Table III, Paper III) the deviations of that predicted by the two theories were substantial and experimentally distinguishable. The typical accuracy by which it is possible to determine steady-state anisotropy values is within $\pm 0.005$ for modern spectrometers, at least for fluorophores exhibiting moderate to high quantum yields of fluorescence. The influence of $A$ on the steady-state
anisotropy and $\Phi_{fr}$ has also been studied. Such procedure will indirectly demonstrate the influence of the Kubo-number. For the two examined configurations, the discrepancy between the values predicted by the EFT and the FT increases with increasing $\Lambda$ and reaches a maximum deviation for Kubo numbers of about unity.

To conclude, the steady-state anisotropy and $\Phi_{fr}$ data obtained with the EFT and the FT, displayed in Table 4.1-4.2, do appear similar but evidently there is a dependence on the coupling strength. Therefore, it is important to account for the dynamics and the local order, something that has been underlined throughout this thesis. The time-resolved depolarisation data predicted for the configurations $\mathcal{O} = UI – UIII$ and the cases 1-5 presented in Fig. 3 in Paper III exemplifies the impact of these properties.

<table>
<thead>
<tr>
<th>Case</th>
<th>$\mathcal{O}$</th>
<th>$\langle \kappa^2(\mathcal{O}) \rangle$</th>
<th>Rotational Correlation Times</th>
</tr>
</thead>
<tbody>
<tr>
<td>R2</td>
<td>isotropic</td>
<td>2/3</td>
<td>$\phi_\alpha = \phi_\beta = \tau_{\alpha 2} = \tau_{\beta 2} = 5\text{ns}$</td>
</tr>
<tr>
<td>R5</td>
<td>isotropic</td>
<td>2/3</td>
<td>$\phi_\alpha = \tau_{\alpha 2} = 5\text{ns}$ $\phi_\beta = \infty$</td>
</tr>
<tr>
<td>U2</td>
<td>(45°,58°,29°) = I</td>
<td>0.35</td>
<td>$\phi_\alpha = \phi_\beta = \tau_{\alpha 2} = \tau_{\beta 2} = 5\text{ns}$</td>
</tr>
<tr>
<td>U5</td>
<td>(45°,58°,29°) = I</td>
<td>0.35</td>
<td>$\phi_\alpha = \tau_{\alpha 2} = 5\text{ns}$ $\phi_\beta = \infty$</td>
</tr>
<tr>
<td>U2</td>
<td>(40°,40°,11°) = II</td>
<td>0.72</td>
<td>$\phi_\alpha = \phi_\beta = \tau_{\alpha 2} = \tau_{\beta 2} = 5\text{ns}$</td>
</tr>
<tr>
<td>U2</td>
<td>(41°,12°,71°) = III</td>
<td>1.60</td>
<td>$\phi_\alpha = \phi_\beta = \tau_{\alpha 2} = \tau_{\beta 2} = 5\text{ns}$</td>
</tr>
<tr>
<td>U5</td>
<td>(41°,12°,71°) = III</td>
<td>1.60</td>
<td>$\phi_\alpha = \tau_{\alpha 2} = 5\text{ns}$ $\phi_\beta = \infty$</td>
</tr>
</tbody>
</table>

Table 4.1 A comprised table of different cases studied in Paper III. The local orientation of the interacting fluorophores ($\alpha$, $\beta$) are either random (R) or uniaxially anisotropic (U).
<table>
<thead>
<tr>
<th>Case &amp; Ω</th>
<th>λ</th>
<th>Φ_Fr (EFT)</th>
<th>Φ_Fr (FT)</th>
<th>r</th>
<th>r_FT</th>
<th>r₀</th>
</tr>
</thead>
<tbody>
<tr>
<td>R2</td>
<td>0.300</td>
<td>0.897</td>
<td>0.880</td>
<td>0.187</td>
<td>0.181</td>
<td>0.206</td>
</tr>
<tr>
<td>R5</td>
<td>0.300</td>
<td>0.901</td>
<td>0.880</td>
<td>0.253</td>
<td>0.240</td>
<td>0.303</td>
</tr>
<tr>
<td>U2I</td>
<td>0.578</td>
<td>0.902</td>
<td>0.847</td>
<td>0.299</td>
<td>0.280</td>
<td>0.319</td>
</tr>
<tr>
<td>U5I</td>
<td>0.578</td>
<td>0.907</td>
<td>0.847</td>
<td>0.328</td>
<td>0.300</td>
<td>0.360</td>
</tr>
<tr>
<td>U2II</td>
<td>0.278</td>
<td>0.903</td>
<td>0.885</td>
<td>0.309</td>
<td>0.307</td>
<td>0.319</td>
</tr>
<tr>
<td>U2III</td>
<td>0.041</td>
<td>0.941</td>
<td>0.969</td>
<td>0.300</td>
<td>0.309</td>
<td>0.319</td>
</tr>
<tr>
<td>U2III</td>
<td>0.125</td>
<td>0.889</td>
<td>0.927</td>
<td>0.280</td>
<td>0.293</td>
<td>0.319</td>
</tr>
<tr>
<td>U2III</td>
<td>2.41</td>
<td>0.796</td>
<td>0.801</td>
<td>0.227</td>
<td>0.230</td>
<td>0.319</td>
</tr>
<tr>
<td>U5III</td>
<td>0.125</td>
<td>0.892</td>
<td>0.927</td>
<td>0.308</td>
<td>0.323</td>
<td>0.360</td>
</tr>
</tbody>
</table>

*Table 4.2* A comprised table of the different cases studied in Paper III. Φ_Fr is the relative quantum yield, r is the steady-state anisotropy predicted by EFT and r₀ is the steady-state anisotropy in absence of energy migration.
5. Conclusions and future outlook

This thesis presents a new theoretical treatment aimed for quantitative analysis of DAET and PDDEM within the framework of the extended Förster theory.

The reorientational motions of the fluorophores have been included in the model of fluorescence relaxation in the presence of PDDEM. Thereby, it is possible to overcome the limitations in the analytical model of PDDEM when the motions occur on the same timescale as the EM and the fluorescence relaxation and hence, increase the informational content in the experimental data. The EFT of PDDEM extends the possibilities of measuring distances between chemically identical fluorophores, which exhibit different photophysical properties e.g. due to their different localisation in a macromolecule. In either theoretical treatment of PDDEM the distance information can be obtained from fluorescence lifetime measurements, provided that the relaxation rates of the two groups are significantly different.

EFT, addresses and surmounts the “κ²-problem”. Additionally, but far from easily accessed, the shape of the fluorescence relaxation and the anisotropy decay of a coupled system between two fluorophores may provide information regarding the mutual configuration in addition to the energy transfer rate.

Hopefully, the theoretical treatment presented here, EFT of PDDEM, will soon also include asymmetrical transfer rates. The theory of energy migration could also potentially be described in the frequency domain. The benefit would be a way to account for the dynamics without the averaging of trajectories.

The theories derived for electronic energy transfer are being experimentally tested on a model system consisting of DA-labelled DNA oligomers. These oligomers have D-groups at two different distances with respect to the fixed A-group position.

The preponderance of the experimental work in fluorescence spectroscopy and in particular fluorescence depolarisation spectroscopy is carried out using one-photon excitation (OPE). Recently, advances regarding energy migration have
been made in the moving field of two-photon excitation (TPE) spectroscopy[29]. In principle, the theories presented in this thesis, could be adopted for TPE.

Although the distance in between the interacting fluorophores have throughout this thesis always been considered to be constant, models of static and dynamic distributions of distances can be included in the EFT. Today, no study has been conducted exploring distance distributions in combination with the EFT. If this information can be separated from $\kappa^2(t)$ is therefore an open question and needs further studies. However, in experimental systems, $\kappa^2(t)$ effectively accounts for eventual effects of distance distributions.

As an extension of EM/ET for intra-molecular distance measurements, described in detail in this thesis, inter-molecular distance measurements can reveal super-molecular structure. The method to examine the spatial organisation of non-covalent polymers using fluorescence labelled monomers has successfully been proven on the structure of filamentous actin[26]. Inter-molecular distance measurements using EM/ET have thus shown to be useful when addressing questions regarding aggregation of proteins. The aggregation is most often either connected to biological function of proteins or to related diseases[30]. Working towards more accurate models of EM/ET can also enhance the resolution when studying of supra-molecular structures.

Development of new methods for incorporating fluorescent probes into macromolecules such as protein structures have been made by several research groups[31-33]. Site-specific incorporation of unnatural fluorescent amino acids would open up new areas of research and are thought to increase the usage of accurate EM/ET methods. With this approach, several difficulties can be avoided. In comparison with the standard procedures of protein labelling[31], perturbation of the protein structure and low labelling efficiency could be circumvented as well as the uncertainties in distance measurements introduced by significant linker lengths between e.g. a DNA backbone and the inserted chromophoric group. Promisingly, combined with site-specific incorporation of unnatural fluorescent amino acids the EFT may become a versatile tool, alongside to nowadays standard NMR and X-ray techniques, especially when facing inadequate concentrations, protein sizes and crystalline qualities.
6. Acknowledgments

First of all, I would like to thank my supervisor Lennart B.-Å. Johansson for his excellent scientific guidance and for all our rewarding discussions.

I would also like to thank my co-supervisor Per-Olof Westlund for invaluable help concerning the mathematical formalism and for his skilled philosophical argumentations.

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I thank the Kempe Foundation and Innovationsbron for financial support and High Performance Computing Center North (HPC2N) for letting me use their resources.

I also thank my parents for all their love and support.

And finally, thanks to all friends for your support and encouragement!
7. Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>acceptor of electronic energy</td>
</tr>
<tr>
<td>BD</td>
<td>Brownian dynamics</td>
</tr>
<tr>
<td>D</td>
<td>donor of electronic energy</td>
</tr>
<tr>
<td>DAET</td>
<td>donor-acceptor energy transfer</td>
</tr>
<tr>
<td>DDEM</td>
<td>donor-donor energy migration</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>EFT</td>
<td>extended Förster theory</td>
</tr>
<tr>
<td>EM</td>
<td>energy migration</td>
</tr>
<tr>
<td>ET</td>
<td>energy transfer/transport</td>
</tr>
<tr>
<td>$F(t)$</td>
<td>time-dependent fluorescence intensity</td>
</tr>
<tr>
<td>FRET</td>
<td>Förster resonance energy transfer</td>
</tr>
<tr>
<td>IC</td>
<td>internal conversion</td>
</tr>
<tr>
<td>IRF</td>
<td>instrumental response function</td>
</tr>
<tr>
<td>ISC</td>
<td>inter-system crossing</td>
</tr>
<tr>
<td>$\kappa^2$</td>
<td>orientation factor</td>
</tr>
<tr>
<td>$\lambda$</td>
<td>wavelength, nm</td>
</tr>
<tr>
<td>NMR</td>
<td>nuclear magnetic resonance</td>
</tr>
<tr>
<td>OPE</td>
<td>one-photon excitation</td>
</tr>
<tr>
<td>PDDEM</td>
<td>partial donor-donor energy migration</td>
</tr>
<tr>
<td>$R$</td>
<td>distance between chromophoric groups</td>
</tr>
<tr>
<td>$R_0$</td>
<td>Förster radius</td>
</tr>
<tr>
<td>RET</td>
<td>resonance energy transfer</td>
</tr>
<tr>
<td>$r(t)$</td>
<td>time-dependent anisotropy</td>
</tr>
<tr>
<td>$S$</td>
<td>order parameter</td>
</tr>
<tr>
<td>SLE</td>
<td>stochastic Liouville equation</td>
</tr>
<tr>
<td>SME</td>
<td>stochastic master equation</td>
</tr>
<tr>
<td>TCSPC</td>
<td>time-correlated single photon counting</td>
</tr>
<tr>
<td>TPE</td>
<td>two-photon excitation or two-photon excited</td>
</tr>
<tr>
<td>$\tau$</td>
<td>fluorescence lifetime</td>
</tr>
<tr>
<td>VR</td>
<td>vibrational relaxation</td>
</tr>
</tbody>
</table>
8. References

2. Förster T. Zwischenmolekulare Energiewanderung und Fluorescenz. Ann Phys (1948) 2, 55-75


