Investigating the distance limit of a metal nanoparticle based spectroscopic ruler

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Conventional Förster resonance energy transfer (FRET) processes involving a pair of fluorophore and organic quencher are restricted to an upper distance limit of ~10 nm. The application of a metal nanoparticle as a quencher can overcome the distance barrier of the traditional FRET technique. However, no standard distance dependence of this resonance energy transfer (RET) process has been firmly established. We have investigated the nonradiative energy transfer process between an organic donor (fluorescein) and gold nanoparticle quencher connected by double stranded (ds) DNA. The quenching efficiency of the gold nanoparticle as a function of distance between the donor and acceptor was determined by time-resolved lifetime analyses of the donor. Our results showed a $1/d^4$ distance dependence for the RET process for longer distances (>10 nm) and $1/d^6$ distance dependence for shorter distances (<10 nm). Our results clearly indicate the applicability of metal nanoparticle based quenchers for studying systems that exceed the 10 nm FRET barrier.

OCIS codes: (170.6280) Spectroscopy, fluorescence and luminescence; (160.4236) Nanomaterials

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1. Introduction

An investigation of molecular interactions and conformational changes of biomolecules such as proteins and nucleic acids is imperative to understand their structural and functional properties [1–5]. For instance, the conformational dynamics of DNA (Deoxyribonucleic acid) play a significant role in regulating cellular functions as well as modulating the sensitivity and selectivity of DNA-based sensors, promising diagnostic devices to decipher the genetic basis of diseases [6]. Förster resonance energy transfer (FRET), a fluorescence-based "spectroscopic ruler" technique [1], involves the nonradiative energy transfer between a pair of organic donor and acceptor molecules and is an attractive optical method to probe distance-dependent structural properties of a molecular system [1–3]. However, the application of FRET to study large macromolecules is restricted due to an upper distance limit of ~10 nm [1,7]. Recently, the use of metal nanoparticles as an acceptor in the energy transfer process has been claimed to surmount the distance-barrier of the conventional FRET method, offering a promising alternative to investigate conformational changes of macromolecules [8–10] Although the resonance energy transfer (RET) between the donor fluorophore and the acceptor nanoparticle takes place at a longer distance, no standard rule for its distance dependence has been established [11–13].

The conventional FRET process is based primarily on the rate of the nonradiative energy transfer between donor and acceptor molecules, appropriately tagged with a biomolecule of interest [1–3]. Owing to excitation, energy emitted from the donor molecule is transferred to the acceptor through distance-dependent dipole-dipole coupling. The selection of a specific donor-acceptor system in the FRET is fundamentally dependent on the overlap of the emission band of the donor fluorophore with the excitation band of the acceptor molecule. The energy transfer process is controlled by the spatial proximity of the donor and acceptor. Any perturbation in the conformation of the biomolecule causes an alteration in the distance between the donor and acceptor, and consequently influences the energy transfer process. Thus, FRET can be utilized to elucidate dynamic conformational changes of biomolecules in microscopic detail [1–4,14]. The FRET process, which follows a $1/d^6$ distance dependence, is regulated by the electromagnetic coupling of two dipoles involved in the conventional organic donor–acceptor system. Thus, application of FRET to study large macromolecules suffers from the spatial limitation of ~10 nm [7,8]. Metal nanoparticles have been used as a promising acceptors to overcome this distance barrier of the FRET measurement [6]. The application of metal nanoparticles as acceptors in the FRET method has significantly improved the quantum efficiency of the energy transfer process to probe a comparatively larger conformational change of a macromolecule, which, until now, has been out of reach of the conventional

FRET technique involving an organic acceptor molecule [7,8]. The primary reason for the enhanced sensitivity of the energy transfer process is attributed to the electromagnetic interaction between the dipole of the donor fluorophore and the surface electrons of the metal nanostructure [7,8], which can take place at a longer distance compared to dipole-dipole coupling. Since the electronic distribution of a metallic nanoparticle is influenced by its size and shape, a suitably controlled nanoparticle can modulate the energy transfer process when it is placed in the vicinity of the donor molecule [7,8]. Furthermore, the orientation of the electronic dipole of the donor with respect to the distance vector between the donor and nanoparticle leads to an alteration in the efficiency of the energy transfer process. Recent theoretical studies claimed that the distance-dependence of the resonance energy transfer (RET) involving the metal nanoparticle and organic donor fluorophore could vary depending on the conditions of the energy transfer process [13]. The ratio of the size of the nanoparticle and the distance vector between the donor and acceptor plays a crucial role in the energy transfer mechanism [13]. The interaction between nanoparticle and organic fluorophore varies with distance between the donor-acceptor pair. At a shorter distance (< 20 Å), radiative enhancement causes an increase of the spontaneous emission rate of donors that are placed in the proximity of metal nanostructures where the density of photonic states is higher than in a homogeneous medium [14,15]. Enhanced rates of spontaneous emission can lead to the reduction of the excited-state lifetime of the emitter. At an intermediate distance (20-300Å), nonradiative energy loss of the donor is a predominant process [7,8]. The nonradiative energy-transfer process varies as f/d^n, where f is the fluorophore's oscillator strength, d is the distance between the donor and the metal surface, and n depends on geometric factors [1]. Recent theoretical studies have attempted to uncover the nonradiative energy transfer mechanism between metal nanoparticles and organic fluorophores [7,8,10–12,16]. The suggested explanations regarding the reported deviation of the energy transfer process between metal nanoparticles and organic donors from the conventional FRET process are attributed to the breakdown of point dipole approximations, insufficient orientation averaging during the lifetime of the donor, and excitation of electron-hole (e-h) pairs in the nanoparticles [10–13,16]. Quantum mechanical studies predicted the rate of the energy transfer process from a fluorescent dye to a spherical nanoparticle might follow a variable distance dependence as $1/d^n$, with $n = 3,4$ at intermediate distances, and Förster's $1/d^6$ dependence could be regarded at large separations between the donor and nanoparticles [11]. Additionally, the predicted energy transfer rate showed an asymptotic, nontrivial nanoparticle size dependence and the orientation factor varied from 1 to 4, contrasting with the traditional FRET process [11]. The use of a spherical jellium model to validate the rate of the nonradiative energy transfer process from the excited fluorescein to the gold nanoparticle has revealed that primary contributions to the energy transfer process originate from the $1/d^6$ term at the distances <28 Å [16]. It has been suggested that the excitation of plasmons or electron-hole pairs of the nanoparticle are not sufficient to explain the energy transfer rate between the donor and nanoparticles at a longer distance [16]. A recent development of generalized Förster theory incorporating distance and torsional fluctuations pointed out that the deviation of the energy transfer process between metal nanoparticle and organic donor could originate from quantum mechanical modulations of donor-acceptor coupling [17]. Considering $n = 4$ for the dipole–metal surface energy transfer process, the characteristic distance (d_0) involving the nonradiative surface energy transfer (SET) between FAM (fluorescein) and gold nanoparticle was estimated to be 76.3 Å [7,8], using the Persson and Lang model [18]. However, the gold nanoparticle is assumed to be an infinitely wide plane of dipoles and the true n value could be slightly greater than 4 in respect of the dipole-surface energy transfer process [19,20]. Recent experimental studies on the surface energy transfer involving DNA conjugated fluorescent dyes and gold nanoparticle system analyzed the experimental results in the light of the Persson and Lang model [18], supporting a $1/d^4$ distance dependence of the energy transfer process [7,8]. Additionally, salt concentration, length of linker molecules connecting the dye and DNA, and

orientation of the fluorescent donor were found to be crucial players for the energy transfer process [19].

In the present study, we investigated the distance–dependent mechanism of the resonance energy transfer process between fluorescein (donor) and gold nanoparticle (acceptor), both attached to DNA, using time-resolved spectroscopic method. Time-resolved spectroscopic studies offer a unique approach to unravel the mechanistic details of the resonance energy transfer process involving a metal nanoparticle as an acceptor under both *in vitro* and *in vivo* experimental conditions [1,3,17,20,21]. The current study investigated primarily the nonradiative energy transfer process beyond the distance regime of the conventional FRET process. The quenching efficiency of the DNA gold nanoparticle as a function of distance between the donor and acceptor was determined by the time-resolved lifetime analyses of the donor molecule. A comparative analysis between RET and conventional FRET methods was performed to validate the enhanced efficiency of the RET mechanism involving a gold nanoparticle as the acceptor.

2. Experimental Method

FAM modified (5′-C_6- FAM) oligostrands were purchased from IDT DNA Technologies. 6-FAM (Fluorescein), which is a single-isomer derivative of fluorescein, is generally used in the pH range 7.5-8.5 as a fluorescent label and can be attached to the oligonucleotide. Commercially obtained gold nanoparticles (1.4 nm diameter) from Nanoprobes Inc. were attached to the complementary thiol labeled (HS-C_3) DNA strands. The resultant gold nanoparticle–dye conjugated double stranded (ds) DNA were prepared and purified following the experimental method reported in Ref. [23]. For all steady state and time-resolved fluorescence measurements, 60 nM DNA solutions were prepared using TE buffer (Sigma Cat# 93302), maintaining the pH of the solution at 7.5. The steady-state fluorescence spectra were acquired using a spectrometer with a Xenon lamp as an excitation source at 367 nm. The time-resolved fluorescence measurements were carried out using a time correlated single photon counting (TCSPC) system (Horiba Jobin-Yvon) at room temperature. A 50 ps diode laser operating at 1 MHz repetition rate and 467 nm emission wavelength was used as the excitation source. The time-resolved data analysis was performed following a deconvolution technique using the iterative nonlinear least squares method. To examine " the "goodness of fit", a perfect agreement between the data and the anticipated model with a specific set of fit parameters was assessed when the normalized χ^2 value (χ^2_R) was close to 1; a value less than 1.2 was considered to be acceptable.

3. Results and Discussion

The steady-state emission measurement of FAM attached to DNA showed a characteristic emission maximum at 518 nm with a full width at half maximum of ~30 nm (Fig. 1). The time-resolved photo luminescence (PL) lifetime measurement of the FAM-DNA system at the emission maximum showed a single exponential decay with an average lifetime of 4.18 ns ($\pm$ 0.02 ns) (Fig. 1). These results confirmed that FAM remained as a stable monomer in the solution at the pH 7.5 [22], without forming a dimer or higher aggregate under the experimental conditions discussed herein.

Time-resolved fluorescence measurements were performed to investigate a distance-dependent quenching process between the donor FAM and the acceptor gold nanoparticles, separated by double stranded DNA with an increasing number of base pairs. Since the persistence length of double stranded DNA is about 50 nm (~150 bp) [24], short DNA strands can be considered as rigid rods. The calculated separation distance assumes a linear DNA strand, with a C_6 spacer between the DNA and the donor and a C_3 spacer with thiol linkage
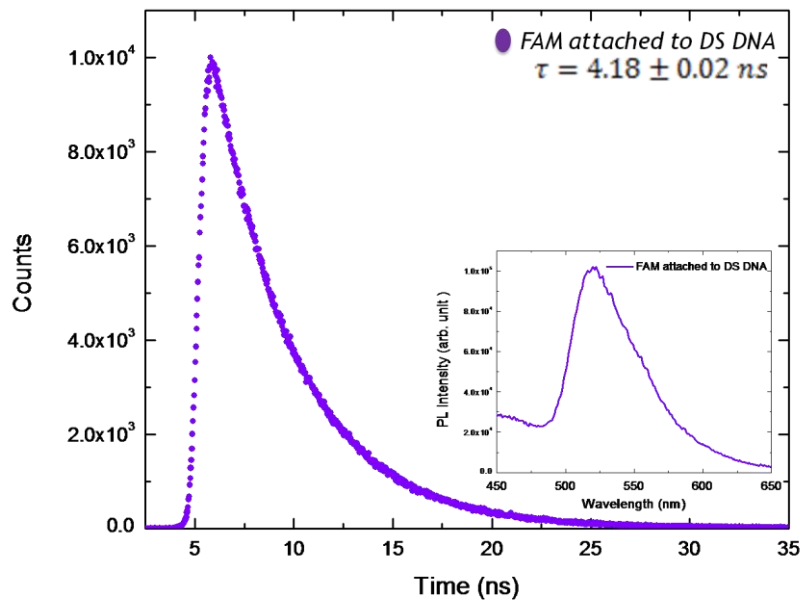
Fig. 1. Time-resolved emission dynamics of FAM exhibiting a single exponential decay with lifetime of 4.18 ns . Steady-state PL spectrum of FAM conjugated with DNA showing the emission maximum at 518 nm (Inset).
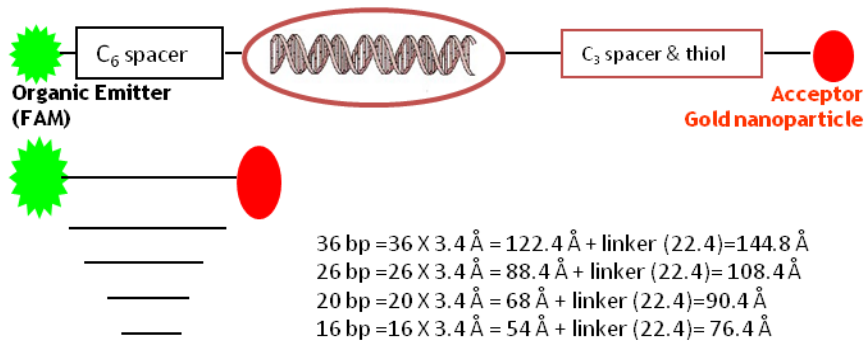

Fig. 2. A schematic drawing of the system under investigation. A 1.4 nm gold nanoparticle and a FAM donor are attached to the two ends of a double stranded DNA via linkers. Four different lengths investigated in the present study are also indicated.

connecting the acceptor gold nanoparticle to the DNA strand (Fig. 2). The measured lifetimes of 16 bp, 20 bp, 26 bp, and 36 bp fragments were 3.08 ± 0.04 ns, 3.16 ± 0.05 ns, 3.84 ± 0.04 ns, and 3.96 ± 0.03 ns, respectively (Fig. 3).

The quenching efficiencies (Q_{eff}) and energy transfer rates (k_{ET}) were calculated following equations Eqs. (1) and (2), respectively, by comparing the measured lifetimes of the quenched fluorophore (τ') with the fluorophore's lifetime (τ_0) in the absence of gold nanoparticle in the identical DNA conjugated system.

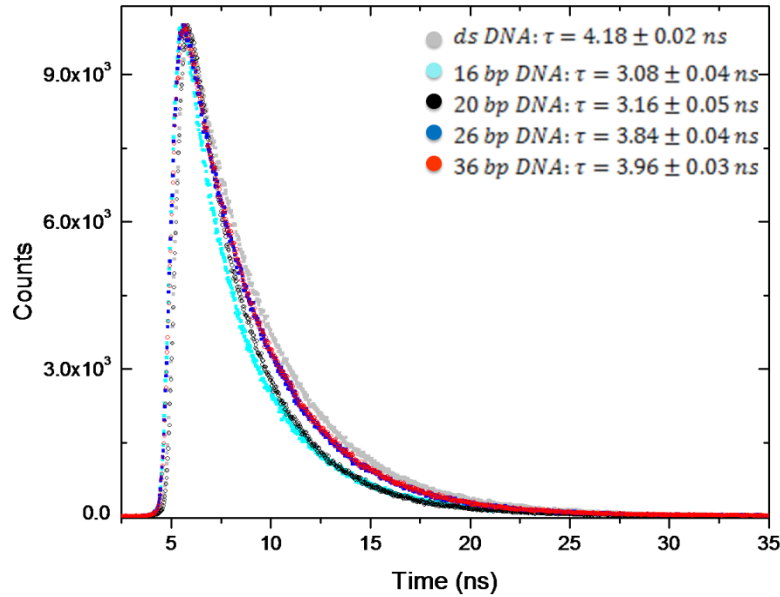$$Q_{eff} = 1 - \frac{\tau'}{\tau_0} \qquad (1)$$

Fig. 3. Results of time-resolved luminescence measurements indicating the change in lifetime observed for the four different distances studied (16 bp, 20 bp, 26 bp and 36 bp).

$$k_{ET} = \frac{1}{\tau'} - \frac{1}{\tau_0} \tag{2}$$

The quenching efficiency of the gold nanoparticle gradually decreased with an increase in the length of the DNA strands. Similarly, the nonradiative energy transfer rate followed a diminishing trend with increasing distance between the donor and acceptor. Our experimental outcomes supported the enhanced quenching ability of the gold nanoparticles at a longer distance compared to the traditional FRET process.

A comparison of quenching efficiencies obtained from our experimental results with a theoretical curve generated from the expression Eq. (3) with d_o value of 70 Å indicated that the energy transfer process involving gold nanoparticle as quencher followed largely a $1/d^4$ distance dependence (Fig. 4) with the distance dependence getting modified at smaller distances. At shorter distances we find the experimental data to follow closer to the traditional FRET dependence of $1/d^6$. The d_o value refers to the separation distance at which the donor will exhibit equal probabilities for energy transfer and spontaneous emission in the presence of an absorber (gold nanoparticles).

$$Q_{eff} = \frac{1}{1 + \left(\dfrac{d}{d_0}\right)^n} \tag{3}$$

Thus, d_o is the distance corresponding to a 50% level of nonradiative energy transfer between the donor fluorescein and acceptor gold nanoparticle.

In summary, this investigation demonstrated the energy transfer between an organic donor and nanoparticle quencher separated by distances greater and within the distance limits of the
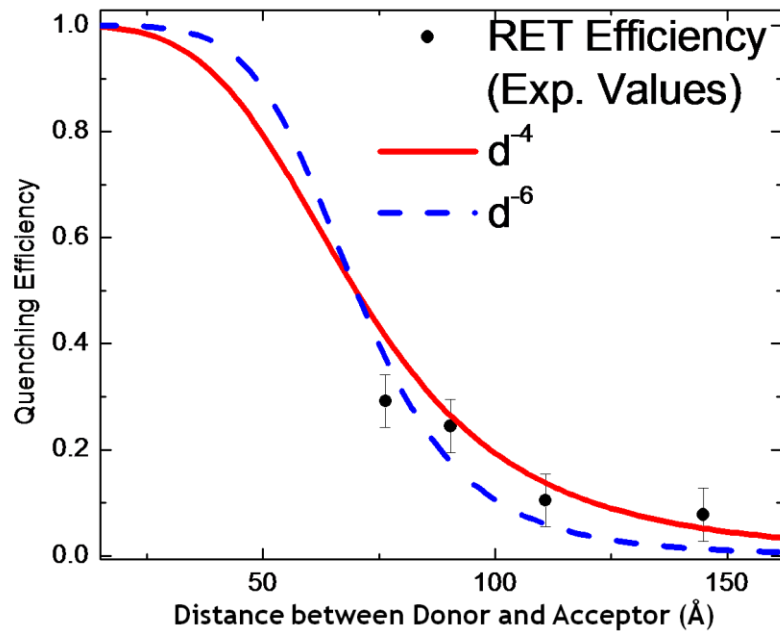
Fig. 4. The quenching efficiency plotted as a function of distance for $1/d^4$ and $1/d^6$ models. At distances greater than 10 nm, the system shows quenching efficiencies closer to the $1/d^4$ model. A d_o value of 70 Å has been used in the above calculations.

conventional FRET technique. We observe two different regimes in the efficiency of the nonradiative energy transfer process depending on the separation. The experimental results presented here confirm that the value of n in the distance dependence $1/d^n$, might indeed be a value that lies between 4 and 6 agreeing with some of the theoretical predictions. Further experimental investigation at even shorter separation distances between the donor fluorophore and nanoparticle is required to unravel the mechanistic details of the nonradiative energy transfer process involving metal nanoparticle quencher.