JOURNAL OF KUFA–PHYSICS
www.uokufa.edu.iq/journals/index.php/jkp | ISSN: 2077–5830

Studying the Fluorescence Resonance Energy Transfer Between Two Dyes of Laser in an Aqueous Solution

Yusser R. Mohammed* **Waled S. Abdullwahab**

Department of physics, College of science, Mustansiriyah University, Iraq.

*Corresponding Author E-mail: Yusser.raed.12@gmail.com

ARTICLE INF

Article history:
Received: 20 JUN, 2022
Revised: 25 AUG. 2022
Accepted: 30 AUG, 2022
Available Online: 10 DEC, 2022

Keywords:

FRET
Acriflavine
Rhodamine B
HPCL Water

ABSTRACT

In the past quarter century, applications of fluorescence resonance energy transfer (FRET) have grown dramatically, and the technology has become an indispensable tool in a wide variety of biological and biophysical domains. It is utilized, in order to acquire information on the conformational changes that occur in single molecules. By utilizing the fluorescence correlation spectroscopy, the pharmaceutical sector has also built huge fluorescence detection systems with extremely small sample sizes, reaching down to the level of single molecules. The fluorescence resonance energy transfer (FRET) between the two dyes, Acriflavine (Acf) and Rhodamine B (RhB), were examined in solution. Energy transfer was observed in fluorescence resonance imaging solutions containing Acriflavine and Rhodamine B with different concentrations of the acceptor RhB dye in the range of (1.5×10^{-5} M to 3.5×10^{-5}M). Studies using Both UV–Vis absorption and fluorescence spectroscopy demonstrated that the two dyes, when dissolved in solution, appear largely as monomers.

DOI: http://dx.doi.org/10.31257/2018/JKP/2022/140201

دراسة نقل طاقة رنين الفلورة بين صبغتين ليزرية في محلول مائي

يسر رائد محمد **وليد صلاح وهاب**

قسم الفيزياء، كلية العلوم الجامعة المستنصرية، بغداد، العراق

الـــخُـــلاصـــة

في ربع القرن الماضي ، نمت تطبيقات نقل طاقة رنين الفلورة (*FRET*) بشكل كبير ، وأصبحت التكنولوجيا أداة لا غنى عنها في مجموعة واسعة من المجالات البيولوجية والفيزيائية الحيوية. يتم استخدامه من أجل الحصول على معلومات حول التغييرات التوافقية التي تحدث في الجزيئات المفردة. من خلال استخدام التحليل الطيفي للارتباط الفلوري ، قام قطاع المستحضرات الصيدلانية أيضًا ببناء أنظمة ضخمة للكشف عن التألق بأحجام عينات

الكلمات المفتاحية:

طبيقات نقل طاقة رنين الفلورة
أكريفلافين
رودامين B
HPCL Water

لى مستوى الجزيئات المفردة. تم فحص نقل طاقة الرنين الفلورة
Acriflavine (Acf) و *Rhodamine B (RhB)*، في المحلول.
ي محاليل التصوير برنين الفلورة المحتوية على *Acriflavine* و
ات مختلفة من صبغة *RhB* المستقبلة في المدى (١.٥ × ١٠-٥ م إلى
أطياف الأمتصاصية والفلورة التي أجريت باستخدام كل من مطياف
المرئية ومطياف الفلورة أن الصبغتين ، عند ذوبانهما في المحلول ،
ى هيئة مونومرات.

1. Introduction

The fluorescence resonance energy transfer (FRET) is a physical mechanism that transfers nonradiatively excited energy from one excited molecular fluorophore (the donor) to another fluorophore through intermolecular long-range dipole–dipole coupling (the acceptor)[1]. In the fields of biology, physiology, medicine, and pharmacology, fluorescent sensors are utilized quite frequently[2]. When it comes to scientific investigation, they have drawn the interest of a great number of chemists and biologists[3]. Utilizing detection methods that are based on fluorescence sensors comes with a number of advantages, such as ease of use, low cost, high sensitivity, quick and simple adaptation to automated analysis, the ability to support spatially resolved images, and a number of different signal output modes[4, 5]. In general, fluorescent sensors offer a one-of-a-kind method for detecting analytes that are relevant from a physiological or ecological perspective[6]. Theodor Forster, who created an equation in 1948 to determine the efficiency of electronic excitation transfer from a donor to an acceptor, is remembered today thanks to the acronym FRET (which stands for his name)[7]. It is possible that FRET is an accurate method for measuring molecule closeness, and it is particularly useful at angstrom distances (10–100)[8]. if the donor and acceptor are within the Forster radius, the donor's excitation energy will be transferred to the acceptor[9]. This distance is typically between 3 and 6 nanometers[10]. FRET efficiency can be affected by a variety of distinct aspects, such as the fluorescence quantum yield of the donor in the absence of an acceptor, the refractive index of the solution, the dipole angular orientation of each molecule, and the spectrum overlap integral of the donor emission and the acceptor absorption, amongst others[11]. If any of these characteristics change as a result of the presence of an outside agent, then the efficiency of energy transfer will change as a result[12]. Because of this, the FRET process has the potential to advance sensor technology[13]. It is essential to discover new FRET pairs since fluorescence spectroscopy has developed into a potent technique for detecting transition and heavy metal ions by investigating and quantifying their FRET mechanism. This study presents the outcomes of our tests on FRET between two dyes, namely acriflavine and rhodamine B [14]. Metal ions are one of those that are mentioned in Metal ions have a substantial impact on both the fluorescence and absorption spectra[15]. It's possible that this will have some kind of effect on the FRET mechanism that occurs between Acr and other dye molecules[11]. As a consequence of this, it is of the utmost importance to carry out research on the FRET between Acf and other dyes under a number of different conditions[16]. The findings indicate that, given a constant concentration of donors, the efficiency of energy transfer improves with decreasing donor concentrations.

2. Experimental

2.1 Materials and methods

Donor and acceptor laser dyes were Acf and RhB, both of which were purchased in

Lancashire, United Kingdom. Acf can be prepared in the form of a powder that is orange or brown. It possesses the chemical formula of C14H14CIN3 and a molecular weight of 259.74 gm/mole, in addition to RhB the chemical formula C28H31ClN2O3 and a molecular weight of 479.02 gm/mole. As a solvent, Milli-Q water with a molecular weight of 18.02 u was utilized. The nature of our dyes might be classified as either cationic RB or anionic Acf. And utilized precisely how it was provided for use.

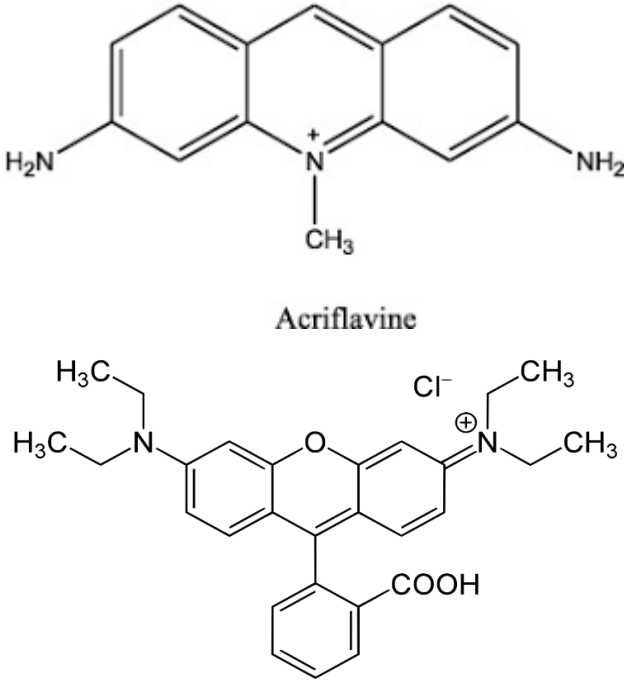

Fig.1: Formulations of curcumin made with chemicals in the Acf and RhB.

According to the following equation, and in order to create the two dyes, a concentration of 1 x 10^{-3} M was used (1)

$$\boldsymbol{m = C\ V\ Mw} \qquad (1)$$

Eq.1. [17] Where: m is the weight of the dye in grams that is required to obtain the desired concentration, C is the concentration that must be prepared, V is the volume of solvent in liters that must be added to the dye, and Mw is the molecular weight of the dye that is being used in g/mol.

The produced dye solutions were diluted in accordance with the equation that is presented below (2)

$$\boldsymbol{C_1\ V_1 = C_2\ V_2} \qquad (2)$$

Eq.2.[18] Where: C1 and C2 refer to the primary and secondary concentrations (M), respectively. Both V_1 and V_2 refer to the volume (in liters) of the solution before and after it has been diluted. Figure 2 shows Acr and RhB dyes in different concentrations 10^{-3}, 10^{-4}, 10^{-5} M .The mixing ratio used for donor and acceptor in this research is (3ml: 3ml)

(a)

(b)

Fig.2: After being diluted with water, the dye rhodamine B (a) and the dye acriflavine (b).

2.2 The measurement of UV–Vis absorption as well as fluorescence spectra

The Ultraviolet Spectrophotometer (T70/T80 series UV/Vis Spectrometer) was utilized in order to take readings for the spectra of UV–Vis absorption as well as steady-state fluorescence. Fluorescence emission was measured using a Spectrofluorophotometer (RF-5301pc Shimadzu). The fluorescence light was collected from the sample surface at an angle of ninety degrees, and the excitation wavelength was 420 nanometers while the sample was at room temperature.

2.3. Theoretical considerations

FACTORS AFFECTING ENERGY TRANSFER RATES

Perrin[19] proposed the use of dipole–dipole interactions as a method by which molecules might interact without colliding at distances greater than their molecular diameters after he solved the mystery that surrounded fluorescence quenching studies, which revealed the occurrence of FRET[20]. This was done after he solved the mystery that surrounded fluorescence quenching studies, which revealed the occurrence of FRET[20]. This was done in order to take into consideration the fact that molecules can interact with one another at distances greater than their molecular diameters without really coming into touch with one another[18]. Forster developed an elegant theory that, by using his famous phrase, offered a quantitative explanation for non-radiative energy transfer[21]. This theory was made possible by Perrin's notion, which Forster relied on[21]. This theory was presented in the context of Forster's presentation.

$$K_T(\mathrm{r}) = \left(\frac{R_0}{r}\right)^6 \frac{1}{\tau_D} \qquad (3)$$

where kT(r) is the known rate of energy transfer from donor to acceptor, τ_D is the donor lifetime in the absence of acceptor, r is the distance between donor and acceptor, and Ro is the ratio of the donor's lifetime to the acceptor's lifetime[18]. The Forster distance, also known as the crucial transfer distance, is the minimum distance between two points at which the rate of energy transfer is equal to the rate of decay[11]. The following expression can be used to determine what the value of R_O will be[22].

$$R_0^6 = \frac{2.07}{128\,\pi^{\circ} N_A} \frac{k^2 Q_D}{n^4} \int_0^{\alpha} F(\lambda)\varepsilon_A(\lambda)\lambda^4\, d\lambda \qquad (4)$$

where F_D is the normalized fluorescence intensity of the donor; $\varepsilon_A(\lambda)$ is the extinction coefficient of the acceptor (in M^{-1} cm^{-1}); is the wavelength (in nm); ϕ_D is the fluorescence quantum yield of the donor when there is no acceptor present [9, 11]; n is the refractive index of the medium; k^2 is the orientation factor of the transition dipole moment between the donor (D) and the acceptor (A)[11]; The spectral overlap integral J(λ) is the name given to the integral that is a part of Equation 2, and its value may be found by using the formula[23]:

$$(\lambda) = \int_0^{\alpha} F(\lambda)\varepsilon_A(\lambda)\lambda^4\, d\lambda \qquad (5)$$

Therefore the above definition of Ro in Eq. (4) can be rewritten in terms of (λ) with units $M^{-1}cm^{1}nm^{4}$as

$$R_{\mathrm{o}} = 0.2108[k^2 n^{-4} \phi_D(\lambda)]\frac{1}{6} \qquad (6)$$

where R_O is expressed in units of A^o E The steady state is a method that can be used to determine the FRET's effectiveness[24].

measurements and is expressed as

$$E = 1 - \frac{F_{DA}}{F_D} \qquad (7)$$

where F_{DA} and F_D refer to the donor fluorescence intensity with and without an acceptor, respectively[25]. F_{DA} stands for donor fluorescence intensity with and F_D stands for donor fluorescence intensity without[6]. The exact distance, r, that separates the donor and the acceptor can then be calculated using

$$r = R_o \left[\left(\frac{1}{E} - 1\right)\right]^{\frac{1}{6}} \qquad (8)$$

In this case, Egs were used to calculate the values of (λ), Ro, E, and r. (4) – (8).

When the donor's fluorescence quantum yield (D) was estimated in the absence of an acceptor, the value of 0.91 was discovered to be associated with pure fluorine when it was dissolved in water[26]. The number that was determined to be quite near to the value that was specified for fluorine was found. In addition to the angles created by these two dipole moments and the vector joining their centers, the angle between the transition dipole moments of D and A molecules has an effect on the orientation factor k^2, which in principle can take on any value between 0 and 4[27]. When the dipoles are oriented in such a way that they are perpendicular to one another, k^2 equals zero, k^2 equals four, k^2 equals two-thirds (when both dyes are spinning freely), and k^2 equals 0.47 (when the dipoles are collinear) (in the case of solid films in which the dipole moments of individual molecules are orientational but do not rotate independently).

$k^2 = 2/3$ (in the case of the dipole moments, individual molecules are orientational and spin by themselves). The value of the medium's refractive index (n) was also utilized based on the references. A water solution has a pH value of 4/3[11, 25].

3. Results and discussion

Figure 3 demonstrates. the normalized UV–Vis absorption and steady-state fluorescence spectra of Pure Acf and RhB in aqueous systems. Monomer features can be seen in both the absorption and the fluorescence spectra[28]. The excitation wavelength of 420 nm was used for the purpose of searching for donor and acceptor energy transfer measurements. Also, this length was the best wavelength to excite the fluorescence spectra. This particular wavelength was decided upon because, at this particular frequency, the amount of RhB that may be absorbed is practically nonexistent. We note from Figure 4 that the concentration of Acr 10^{-5} M is fixed and rhodamine the best concentration of energy transfer is $3.5 * 10^{-5}$ M. For the purpose of the energy transfer mechanism, because this concentration was found to be one of the most effective concentrations when mixed with varying concentrations of the acceptor RhB dye in the range of 1.5×10^{-5}M to 3.5×10^{-5}M Figure 5. The wavelength of absorption was chosen in order to come as close as possible to simulating direct flu molecule excitation while simultaneously avoiding or minimizing direct flu molecule excitation. The RhB molecules are made active. Figure 5a illustrates the fluorescence spectra of pure Acf and RhB, as well as their mixtures in aqueous solution (50:50 volume ratios). Figure 5b demonstrates that Acf has its own unique fluorescence band. The RhB fluorescence band, on the other hand, is not very noticeable in dye solution. The more concentration the RhB, the lower the fluorescence. It is essential to take into account the fact that the intensity of the RhB fluorescence increases while the intensity of the Acf fluorescence drops when the concentration is held constant at 10 M. In this instance. As a consequence of this, it achieves its highest value as energy transfer at a concentration for acceptor of 3.5×10^{-5}M with a concentration of the donor that remains constant.

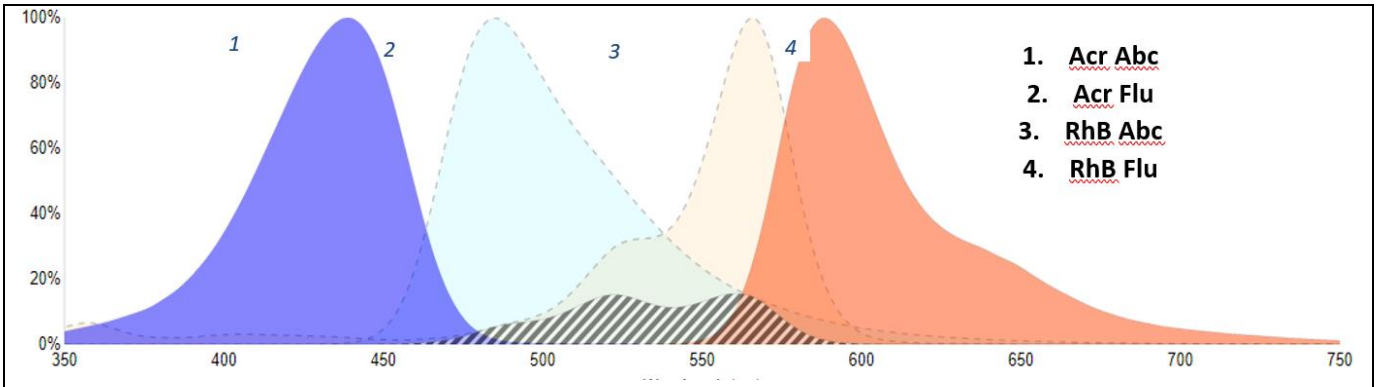

Fig. 3: Normalized UV–Vis absorption and fluorescence spectra of Acr and RhB in aqueous solution. The overlap between Acr fluorescence (2) and RhB absorption (3) spectra is shown by shaded region.

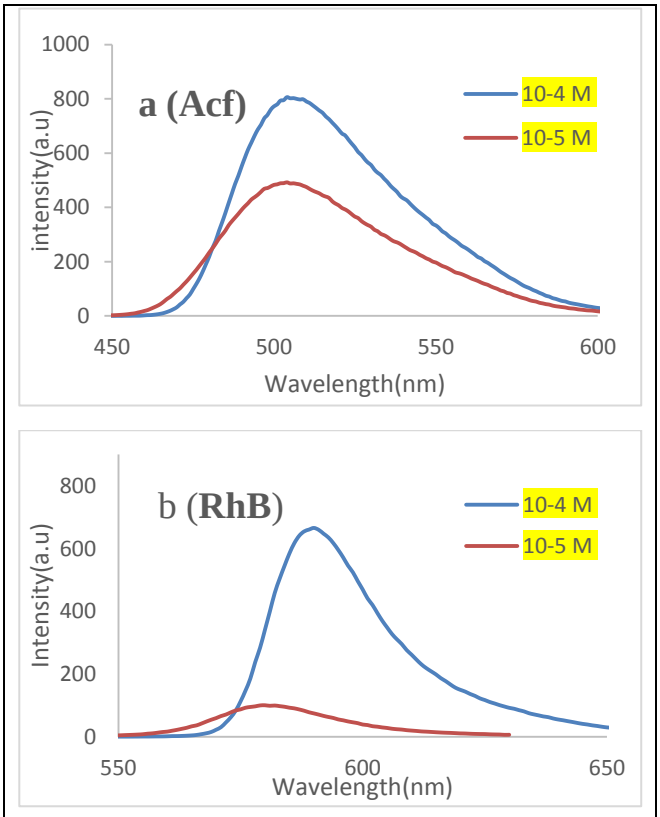

Fig.4: (a) The fluorescence spectra of Acf at concentrations of $10^{-4}M$ (1) and $10^{-5}M$ (2). (b) The concentrations of RhB at $10^{-4}M$ (1) and $10^{-5}M$ (2). Excitation wavelength of 420 nm was used for the measurement of each spectrum.

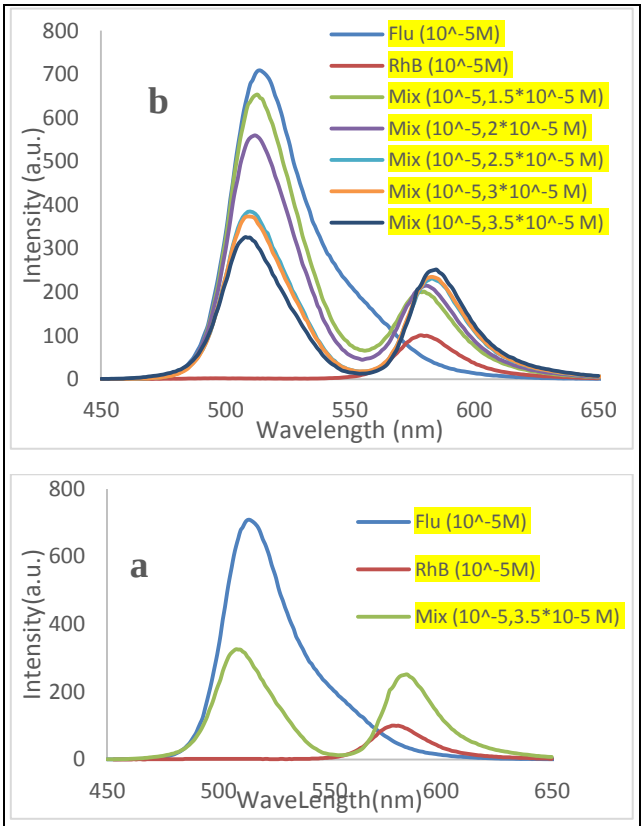

Fig. 5: (a) Fluorescence spectra of RhB (3), Acf (1), and Acf+RhB (50:50) mixture (2) in aqueous solution. RhB concentration was 10^{-5} M; Acf concentration was 10^{-5}M. (b) Fluorescence spectra of a mixture of Acf and RhB with varying acceptor concentrations for a constant amount of donor. The effectiveness of the FRET process is shown below as a function of the acceptor concentration. Excitation wavelength of 420 nm was used for all of the spectra's measurements.

Table 1: presents the values of the spectral overlap integral J(λ), the Forster radius (Ro), the donor–acceptor distance (r), and the energy transfer efficiency (E %) for FRET between Acf and RB at various acceptor concentrations in aqueous solution. The concentration of the donor was maintained at 10-5 M throughout the experiment (these values were derived from the spectral properties shown in Fig.3) (Supporting information).

Acceptor (RB) concentraction (in M)	$J(\lambda) \times 10^{16}$ $M^{-1}cm^{-1}nm^4$	E (%)	R (nm)	r(nm)
$1.5*10^{-5}$	1.91	0.24	6.48	7.79
$2*10^{-5}$	2.08	0.35	6.57	7.25
$2.5*10^{-5}$	2.23	0.55	6.64	6.43
$3*10^{-5}$	2.27	0.56	6.66	6.4
$3.5*10^{-5}$	2.38	0.62	6.71	6.2

4. Conclusions

The term "FRET" refers to the process through which energy is transferred between two fluorescent dyes. Research on Acriflavine and Rhodamine B in solution has been carried out to a satisfactory level. Experiments with UV-Vis absorption and fluorescence spectroscopy show that, and that the Acf and RhB absorption spectra overlap sufficiently to allow FRET from Acf to RhB. Additionally. There was a transfer of energy, The efficiency of the solution was greater with the mixed dye system, which consisted of 50% Acf and 50% RhB. . The observed energy transfer was at the concentration 3.5 * 10^{-5} M, which is considered to be the best concentration and which had the transfer efficiency (0.62%). Another observation we can make based on the results is that the shapes of the pigments change according to the concentrations before and after the energy transfer.

5. REFERENCES

[1] A. Das, C. Corbella Bagot, E. Rappeport, T. Ba Tis, and W. Park, "Quantitative modeling and experimental verification of Förster resonant energy transfer in upconversion nanoparticle biosensors," Journal of Applied Physics, vol. 130, no. 2, p. 023102, 2021.

[2] S. Chatterjee and A. K. Kar, "Oxygen-Vacancy-Dependent Photocatalysis for the Degradation of MB Dye Using UV Light and Observation of Förster Resonance Energy Transfer (FRET) in PANI-Capped ZnO," The Journal of Physical Chemistry C, vol. 124, no. 33, pp. 18284-18301, 2020.

[3] A. Kaur, P. Kaur, and S. Ahuja, "Förster resonance energy transfer (FRET) and applications thereof," Analytical Methods, vol. 12, no. 46, pp. 5532-5550, 2020.

[4] G. Chen, F. Song, X. Xiong, and X. Peng, "Fluorescent nanosensors based on fluorescence resonance energy transfer (FRET)," Industrial & Engineering Chemistry Research, vol. 52, no. 33, pp. 11228-11245, 2013.

[5] J. Enderlein, "Modification of Förster Resonance Energy Transfer Efficiencyat Interfaces," International Journal of Molecular Sciences, vol. 13, no. 11, pp. 15227-15240, 2012.

[6] D. Dey, J. Saha, A. D. Roy, D. Bhattacharjee, and S. A. Hussain, "Development of an ion-sensor using fluorescence resonance energy transfer," Sensors and Actuators B: Chemical, vol. 195, pp. 382-388, 2014.

[7] H. P. de Oliveira and M. H. Gehlen, "Electronic energy transfer between fluorescent dyes with inter-and intramicellar interactions," Chemical physics, vol. 290, no. 1, pp. 85-91, 2003.

[8] R. M. Clegg, A. I. Murchie, A. Zechel, and D. Lilley, "Observing the helical geometry of double-stranded DNA in

solution by fluorescence resonance energy transfer," Proceedings of the National Academy of Sciences, vol. 90, no. 7, pp. 2994-2998, 1993.

[9] C. L. Hawkes, "Competency-based versus task-based job descriptions: Effects on applicant attraction," 2013.

[10] W. R. Algar et al., "Quantum dots as simultaneous acceptors and donors in time-gated Forster resonance energy transfer relays: characterization and biosensing," Journal of the American Chemical Society, vol. 134, no. 3, pp. 1876-1891, 2012.

[11] G. Li, G. Liu, D.-B. Zhang, and S.-Z. Pu, "A new fluorescence probe based on fluorescein-diarylethene fluorescence resonance energy transfer system for rapid detection of Cd2+," Tetrahedron, vol. 72, no. 41, pp. 6390-6396, 2016.

[12] H. Sahoo, "Förster resonance energy transfer–A spectroscopic nanoruler: Principle and applications," Journal of Photochemistry and Photobiology C: Photochemistry Reviews, vol. 12, no. 1, pp. 20-30, 2011.

[13] T. Cordes et al., "Sensing DNA opening in transcription using quenchable Forster resonance energy transfer," Biochemistry, vol. 49, no. 43, pp. 9171-9180, 2010.

[14] B. Herman, R. V. Krishnan, and V. E. Centonze, "Microscopic analysis of fluorescence resonance energy transfer (FRET)," Protein-Protein Interactions, pp. 351-370, 2004.

[15] J. Zhou, J. Chen, Y. Ge, and Y. Shao, "Two-dimensional nanomaterials for Förster resonance energy transfer–based sensing applications," Nanophotonics, vol. 9, no. 7, pp. 1855-1875, 2020.

[16] J. Szöllosi, S. Damjanovich, and L. Mátyus, "Application of fluorescence resonance energy transfer in the clinical laboratory: routine and research," Cytometry: The Journal of the International Society for Analytical Cytology, vol. 34, no. 4, pp. 159-179, 1998.

[17] E. Lee, C. Kim, and J. Jang, "High-Performance Förster Resonance Energy Transfer (FRET)-Based Dye-Sensitized Solar Cells: Rational Design of Quantum Dots for Wide Solar-Spectrum Utilization," Chemistry–A European Journal, vol. 19, no. 31, pp. 10280-10286, 2013.

[18] M. Krumer-Nevo, I. Weiss-Gal, and L. Levin, "Searching for poverty-aware social work: Discourse analysis of job descriptions," Journal of Social Policy, vol. 40, no. 2, pp. 313-332, 2011.

[19] M. H. Jacob, R. N. Dsouza, I. Ghosh, A. Norouzy, T. Schwarzlose, and W. M. Nau, "Diffusion-enhanced forster resonance energy transfer and the effects of external quenchers and the donor quantum yield," The Journal of Physical Chemistry B, vol. 117, no. 1, pp. 185-198, 2013.

[20] A. K. Kenworthy, "Imaging protein-protein interactions using fluorescence resonance energy transfer microscopy," Methods, vol. 24, no. 3, pp. 289-296, 2001.

[21] J. Saha et al., "Investigation of fluorescence resonance energy transfer between fluorescein and rhodamine 6G," Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, vol. 149, pp. 143-149, 2015.

[22] S. Ganesan, S. M. Ameer-Beg, T. T. Ng, B. Vojnovic, and F. S. Wouters, "A

dark yellow fluorescent protein (YFP)-based Resonance Energy-Accepting Chromoprotein (REACh) for Förster resonance energy transfer with GFP," Proceedings of the National Academy of Sciences, vol. 103, no. 11, pp. 4089-4094, 2006.

[23] L. Voith von Voithenberg and D. C. Lamb, "Single pair Förster resonance energy transfer: a versatile tool to investigate protein conformational dynamics," BioEssays, vol. 40, no. 3, p. 1700078, 2018.

[24] R. B. Sekar and A. Periasamy, "Fluorescence resonance energy transfer (FRET) microscopy imaging of live cell protein localizations," The Journal of cell biology, vol. 160, no. 5, p. 629, 2003.

[25] R. M. Clegg, A. I. Murchie, A. Zechel, C. Carlberg, S. Diekmann, and D. M. Lilley, "Fluorescence resonance energy transfer analysis of the structure of the four-way DNA junction," Biochemistry, vol. 31, no. 20, pp. 4846-4856, 1992.

[26] J. S. Lindsey, M. Taniguchi, D. F. Bocian, and D. Holten, "The fluorescence quantum yield parameter in Förster resonance energy transfer (FRET)—Meaning, misperception, and molecular design," Chemical Physics Reviews, vol. 2, no. 1, p. 011302, 2021.

[27] J. Lei, L. Wang, and J. Zhang, "Ratiometric pH sensor based on mesoporous silica nanoparticles and Förster resonance energy transfer," Chemical communications, vol. 46, no. 44, pp. 8445-8447, 2010.

[28] S. Kuila and S. J. George, "Phosphorescence energy transfer: ambient afterglow fluorescence from water-processable and purely organic dyes via delayed sensitization," Angewandte Chemie International Edition, vol. 59, no. 24, pp. 9393-9397, 2020.