

A Review of Fluorescence Spectroscopic Studies Inside Confined Systems

Chiranjib Ghatak

Department of Chemistry, Jogesh Chandra Chaudhuri College, Kolkata 700033, India

Corresponding Author's Email: c.ghatak79@gmail.com

Abstract

Fluorescence spectroscopic techniques, when combined with advanced analytical tools, serve as fundamental methods across physics, chemistry, and biology. Fluorescence imaging capable of detecting concentrations as low as the nanomolar range demonstrates exceptional sensitivity in examining complex molecular environments and interactions at the single-molecule level. Photophysical events induced by light, including Fluorescence Resonance Energy Transfer (FRET), Photoinduced Electron Transfer (PET), and solvation dynamics, naturally occur in biological systems. However, studies of these photoreactions in solution often differ from observations in their natural contexts. Interfacial or confined reaction centers, located in close cellular proximity, may govern the outcomes of these processes. A comprehensive understanding of mechanistic pathways within confined regions requires extensive investigation using high-quality fluorescence probes within organized assemblies such as micelles, reverse micelles, and vesicles. Distinct local characteristics of the restricted cellular interface—such as reduced degrees of freedom, increased rate constants, enhanced mobility, and altered proton concentrations—compared to bulk solution, likely contribute to these differing results. This paper provides a broad overview of the prevailing effects of organized assemblies on various photophysical and dynamic processes.

Keywords: Fluorescence Spectroscopy.; Micelle; Organised Assemblies; Reverse Micelle

Introduction

The confinement effect can do either or both: shape selectivity for reactants and products and modify the compartmentalization of the encapsulated moieties, thus changing the course of the reaction pathway in a unique way (Das *et al.*, 2018). In this chapter, I am interested in representing the effect of organized assemblies on various light-driven processes by using the fluorometric method, a highly sensitive analytical technique (Bhattacharyya, 2001). Researchers will gather mechanistic views using such ultra-sensitive detection tools about molecular anisotropy, solvation, and rotational relaxation of the probe attached to a lipid or surfactant or protein, etc.; structural or spatial disorderliness; and point-to-point measurement inside nanopools (El-Nahass & El-Gamil, 2026). In a high interfacial region with strict molecular geometry, the light-absorbing moieties in subcellular confinement behave differently compared to bulk solvents. To mimic such unique compartmentalization in a laboratory, researchers must study a wide range of systems composed of various types of molecules with distinct polarity and structural flexibility. Only then can one achieve the heterogeneous nature of the working, organized assembly, like a micellar environment comprising hydrophilic heads and hydrophobic tails. Self-assembling molecular aggregates inside polar solvents (micelles) or non-polar solvents (reverse micelles) and vesicular structures with ionic or non-ionic lipids (liposomes) or surfactants (niosomes) are the wide variety of such systems (Schomäcker & Holmberg, 2009; Kalyanasundaram, 2012). Bengal. The present observation also indicated that pollinators play a role in enhancing the pollination ability, which in turn aims to increase the crop's seed yield.

Objectives

Aqueous moieties present in a nanometer-sized system are capable of controlling the reactivity with the representation of unique smaller pockets of water pools. Very often water molecules dictate the biological

functions of many biomacromolecules when confined inside a biomembrane. I am here reviewing a few photoreactions like FRET, PET, and solvent relaxation studies using self-assembling molecular aggregates inside polar solvents (micelles) or nonpolar solvents (reverse micelles), vesicular structures with ionic or nonionic lipids (liposomes) or surfactants (niosomes), etc. Such restricted geometry with unique structural properties of the building blocks creates non-homogeneous behavior. For visual representation, the schematic figures of micelles, reverse micelles (Figure 1), and multilamellar and unilamellar vesicles were given, and such figures help immensely to understand the heterogeneity of such a system with varying polarity, viscosity, etc.

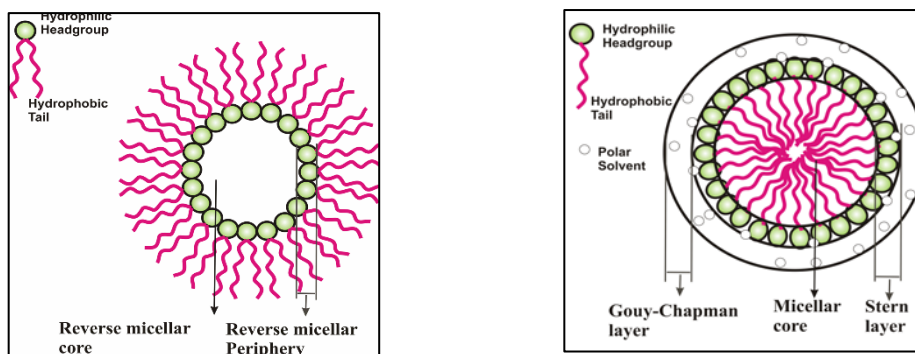


Figure1: Structure of Reverse Micelle (left) and Micelle (right)

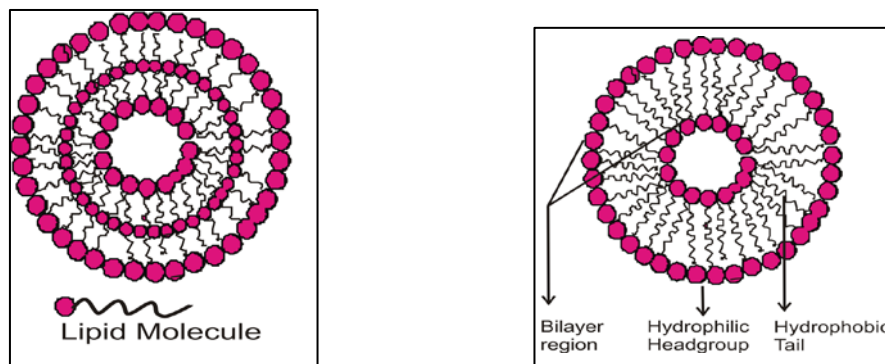


Figure 2: Structure of Multilamellar (left) and Unilamellar Vesicle(right)

FRET Inside Organized Media

In this context, the chapter discusses Förster Resonance Energy Transfer (FRET) within self-assembling molecular aggregates in polar solvents (micelles) and non-polar solvents (reverse micelles), as well as in vesicular structures composed of ionic or nonionic lipids (liposomes, Figure 2) or surfactants (niosomes, Figure 2). It also examines the influence of lipid membrane fluidity on FRET when the donor and acceptor are embedded in small unilamellar vesicles (SUVs) with an approximate diameter of 50 nm. Lipid membranes have a transition temperature (T_m) below which they exist in a solid phase with reduced mobility; above T_m , lipid molecules adopt a more fluid phase with extended hydrocarbon chains. Efficient energy transfer requires donor molecules with high fluorescence quantum yield, while the acceptor—though not necessarily fluorescent—must have a high molar extinction coefficient. FRET occurring over distances of 1–10 nm acts as a “spectroscopic ruler,” being highly sensitive to the inverse sixth power of the donor–acceptor separation (Stryer & Haugland, 1967). The rate of energy transfer is defined by the following equation:

$$k_T(r) = \frac{Q_D \kappa^2 \left(\frac{9000(\ln 10)}{128\pi^5 N n^4} \right)}{\tau_D r^6} \frac{\int_0^\infty F_D(\lambda) \varepsilon_A(\lambda) \lambda^4 d\lambda}{\int_0^\infty F_D(\lambda) d\lambda}$$

Where (N) is Avogadro's number, (n) is the refractive index, (τ_D) is the fluorescence lifetime of the donor in the absence of the acceptor, and (Q_D) is the quantum yield of the donor in the absence of the acceptor. In this equation, ($F_D(\lambda)$) is the emission intensity of the donor, ($\varepsilon_A(\lambda)$) is the molar absorption coefficient of the acceptor, and (κ^2) is the orientation factor, typically taken as 0.67. This approximation for the orientation factor is valid only when dynamic random averaging of the donor and acceptor is considered.

The expression of the overlap integral $J(\lambda)$ is given by the following equation, where one has to have a considerable amount of overlap between the emission spectra of the donor molecule and absorption spectra of the acceptor molecule:

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

Fluorescence intensity of the donor has no dimension. Where the molar absorption coefficient is in $M^{-1}cm^{-1}$ and the wavelength is in nanometers, then the unit of the overlap integral is in units of $M^{-1}cm^{-1}nm^4$. Energy transfer was observed using self-assembling molecular aggregates inside polar solvents (micelles) or non-polar solvents (reverse micelles) (De *et al.*, 2003), and also a few research studies clearly demonstrated an increased FRET rate by many folds compared to that of the bulk medium (Dar *et al.*, 2018), and such an increment of the rate constant inside molecular aggregates was concluded to be for the restrictive nature of the local environment where the donor-acceptor is embedded and is unable to move too far away from each other. Fluorescence resonance energy transfer study in the presence of TX-100 micelle and reverse micelle was investigated by De *et al.* (2003) with a special type of probe: fluorescein, acridine orange, and a neutral probe, Nile red, and similar to Dar *et al.* (2018), a marked increase in the rate constant has been reported inside such a micellar pool/interface (Fang, 2013). Aerosol octyl sulfate-containing reverse micelles and micelles using sodium dodecyl sulfate (SDS), Pluronic copolymer, etc., were used to check the effect of confinement on FRET and were found to have more efficient FRET parameters inside such an organized environment due to the fact that the donor-acceptor couple is closely packed, having optimal proximity to each other (Mondal *et al.* 2006). Other than micelles and reverse micelles, a FRET study was also conducted inside a small unilamellar vesicle with a completely different objective (Ghatak *et al.*, 2011). The search for the effect of temperature on the rate constant of energy transfer was designed for the Coumarin-153–Rhodamine 6G and Coumarin-151–Rhodamine 6G pairs. Using DMPC SUV, results were shown that there was a prominent effect for the Coumarin-153–Rhodamine 6G pair with the change in temperature compared to those of the other FRET pairs. The deeper location of coumarin-153 inside vesicular architecture is the main cause for the improved FRET parameters. Suggestive data in this work also explained that rigidity of the long alkyl chain changes with temperature, which in turn affects FRET parameters in a larger way for the probe, which is deeply localized, i.e., coumarin 153.

Solvation Dynamics inside Organized Media

The vital role of water molecules in dictating structural and dynamical reactivity motivates the study of solvation, or solvent (water) dynamics. In the solvation process, solvent molecules surround solute particles

(ions or molecules). Specifically, solvation dynamics is the study of the time-dependent, non-equilibrium reorganization of polar solvent molecules around a charged probe molecule (Jimenez *et al.*, 1994). Solvation dynamics typically occurs on picosecond to femtosecond time scales and is often triggered by instantaneously formed dipoles through pulse excitation. This process can affect chemical reactions in multiple ways.

Fluorescent probes that show large changes in charge separation between the ground and excited states are commonly used to study solvent relaxation. Since water molecules dictate the biological properties of many biomacromolecules, probing different regions of such assemblies is crucial. This discussion provides a brief overview of solvation dynamics in confined media or interfacial regions.

Compartmentalization within restricted nanopools or interfacial regions drastically alters physical properties such as polarity, anisotropy, mobility, and acidity/basicity (Hansen, 2025; Smortsova *et al.*, 2017). Confined media commonly used in laboratory studies include micelles, microemulsions, lipid bilayers, surfactant bilayers, and multilayers. Micelles formed from negatively charged, positively charged, or neutral surfactants have been used to study solvent relaxation, often showing significantly slower dynamics than in bulk media (Mandal *et al.*, 2002).

Using femtosecond upconversion setups and streak cameras with highly solvatochromic fluorescent probes such as 4-(dicyanomethylene)-2-methyl-6-(p-dimethylaminostyryl)-4H-pyran and coumarin-153, researchers have reported both fast and slower components of solvation dynamics (Mandal *et al.*, 2002; Mondal *et al.*, 2023). Computational studies employing MD simulations, dynamic density functional theory, and QM/MM (Quantum Mechanics/Molecular Mechanics) models allow atomistic analysis of solute environments and solvent reorganization on ultrafast time scales.

The strength and dynamics of hydrogen bonding between water molecules and the polar parts of micelles are significantly slower than hydrogen bonds between water molecules in bulk (Balasubramanian & Bagchi, 2001). Studies comparing interaction energies, hydrogen-bond breaking/forming dynamics, and solvent reorientation lifetimes in bulk solutions versus confined pools have shown much slower solvation dynamics in smaller water pools, such as in n-heptane/AOT/water microemulsions (Sarkar *et al.*, 1996).

Solvation dynamics in microemulsions containing negatively charged, positively charged, or neutral surfactants also exhibit distinctive preferences and complex behaviors, consistent with the heterogeneous nature of such assemblies. Similar slow dynamics have been reported in non-aqueous reverse micelles and vesicles (Corbeil & Levinger, 2003). Differential polarity gradients and microviscosity within lipid molecules containing chromophores in unilamellar and multilamellar structures offer unique confinement environments, which can be explored through solvation studies.

Sýkora *et al.* (2002) reported bimodal lifetimes for water molecules inside vesicles. Key findings indicate that solvation dynamics inside vesicles are significantly slower and more complex due to the presence of water in different localized regions. Excitation wavelength dependence of solvation and rotational relaxation was also observed. For example, three solvent relaxation components (two slow, one fast) were reported in multilamellar vesicles formed with non-ionic surfactants and long-chain polymers (Ghatak *et al.*, 2011). Detailed analysis revealed that the two slow components arise from differing viscosities in the headgroup regions: one in the internal water pool and one in the external bulk-facing region. Local backbone vibrations and solvent-polymer interactions within the bilayer contribute to rapid conformational changes and ultrafast water orientation, producing the fast solvation component.

PET Inside Organized Media

Photoinduced electron transfer is a photophysical incident where, upon excitation with light energy or a photon, the donor moiety causes electron transfer towards the acceptor part, creating a charge separation, and is frequently observed in various natural and synthetic systems. PET can be intramolecular

(same molecule) or intermolecular (different molecule) (Bolton *et al.*, 1991; Romanetz *et al.*, 2026). The theory regarding the electron transfer was first proposed by R. A. Marcus in a seminal paper. PET can happen either through bonds or space, and such a mechanism involves overlap between orbitals of donor and acceptor molecules, which implies that the D-A distance plays a crucial role influencing the rate constant value. The expression for the free energy change (ΔG) for such a PET process can be calculated by using the Rehm-Weller equation:

$$\Delta G = E(D^+/D) - E(A/A^-) - E_{00} - e^2/\epsilon d$$

The first term and the second term on the right side of the equation represent reduction potentials of the donor and the acceptor molecules, respectively. E_{00} is the threshold photoexcitation energy from the singlet ground state to the first excited state, and the last term is the estimated electrostatic interaction between the excited donor and acceptor after charge transfer, where e is 1.6×10^{-19} C, ϵ is the dielectric constant of the medium used, and d is the separating distance.

The rate (k_{ET}) for the kinetics of PET process can be given by,

$$k_{ET} = k_{el} \nu_n \left(\Delta G^* / k_B T \right)$$

K_{el} is the probability or electronic factor that ensures the continuation of the event towards product state and represents the frequency factor. It reflects the Boltzmann factor. ΔG^* is free energy or the driving force.

It is defined as

$$\Delta G^* = \frac{1}{4\lambda} (\lambda + \Delta G^0)^2$$

Now comparing the equations, the classical Marcus equation is obtained

$$K_{ET} = K_{el} \nu_n \exp \left(\frac{-(\lambda + \Delta G^0)^2}{4\lambda K_B T} \right)$$

The reorganization energy (λ) is the sum of solvent reorganization energy and inner-sphere reorganization energy. The dielectric constant, or polarity, of the medium plays a significant role in controlling the activation barrier for electron transfer (ET) by stabilizing the charged transition state. According to Marcus's theory, the relationship between activation free energy, driving force (ΔG^0), and reorganization energy can be summarized as follows:

1. $\lambda > \Delta G^0$: In the normal Marcus region, $\log k$ increases as ΔG^0 decreases.
2. $\lambda = \Delta G^0$: The reaction becomes barrierless.
3. $\lambda < \Delta G^0$: In the inverted region, $\log k$ decreases with increasing driving force.

To study ET, electron-rich molecules such as aniline, N,N-dimethylaniline, N,N-diethylaniline, N,N-dipropylaniline, and N-deuterio-N-ethylaniline are commonly used as donors, while high-electron-affinity molecules like various coumarin dyes (C-151, C-152, C-481, etc.) serve as acceptors (Chakraborty *et al.*, 2008). The choice of donor-acceptor pair and the polarity of the solvent are critical for efficient redox reactions, solar energy conversion, and biological processes.

Given the structural and electronic heterogeneity inside micellar microenvironments, researchers have explored how ET rates and related parameters change in micelles compared to bulk solvent. For instance, studies using negatively charged and nonionic micelles with N, N-dimethylaniline as the donor and oxazine as the acceptor reported significantly slower ET rates than in bulk solution (Pal *et al.*, 1999). This retardation arises primarily from increased donor-acceptor separation within the micellar or interfacial region.

Nanda *et al.* (2005) conducted experiments and theoretical calculations of ET rate constants in micelles composed of SDS, CTAB, and TX-100. They modeled micelles as heterogeneous systems with three distinct regions: a hydrophobic core formed by densely packed alkyl chains, a headgroup shell or interface with intermediate polarity and viscosity, and the surrounding water representing bulk solvent characteristics. Using Marcus theory, they proposed a two-dimensional electron transfer (2DET) model, where ET dynamics depend not only on the driving force but also on solvent reorganization energy. Such ET involves nonequilibrium solvent configurations during rapid electron transfer, and bimolecular diffusion rates were found to be slower than ET rates, indicating that reactant diffusion plays an insignificant role.

These studies revealed a Marcus inversion region, with a maximum rate at lower exergonicity, reflecting the presence of nonequilibrium solvent states in heterogeneous dielectric environments. Although surfactants like CTAB, DTAB, and TTAB form structurally similar micelles, variations in micelle size and shape can significantly influence ET rates. In some cases, researchers calculated reorganization energies for intermolecular photoinduced ET by presuming the location of donors and acceptors within the micellar environment.

Organized assemblies such as micelles, nanoscopic water droplets in organic solvents, and double-layered vesicles often alter donor–acceptor distances and orientations, resulting in faster or slower ET rates. Recently, photoinduced ET (PET) has been applied in cellular imaging and disease therapy (Niu *et al.*, 2023). Studies of PET in vesicular environments indicate that lipid bilayers or multilayers control ET rates through modifications of reorganization free energy, reaction free energy, nuclear dynamics, and donor–acceptor coupling strength. In these systems, donor and acceptor diffusion remains slower than ET, producing a Marcus inversion curve and highlighting the role of nonequilibrium solvation and conformational dynamics (Ghatak *et al.*, 2012).

Conclusion

In this discussion, results on several photophysical phenomena were carefully presented and analyzed, citing numerous relevant studies to highlight the main findings and objectives of the article. It is evident that studying solvation dynamics and electron transfer in a heterogeneous medium at different excitation wavelengths provides insights into solvent dynamics coupled with electron transfer and energy transfer processes in various microenvironments. Such investigations can ultimately enable non-invasive probing of intracellular microviscosity, cell membrane structure, and molecular interactions. Achieving this requires a detailed understanding of the mechanisms underlying these photoinduced events within restricted or interfacial environments.

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