पराद्रुत प्रतिदीप्ति अपकन्वर्जन

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पराद्रुत फोटोइंड्यूस्ड प्रक्रियाओं के यांत्रिकी और गतिज विवरण के अन्वेषण में प्रतिदीप्ति अपकन्वर्जन तकनीक

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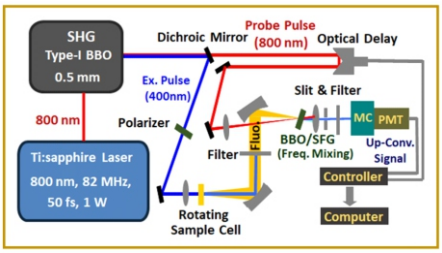

आरपीसीडी, बीएआरसी में विकसित फेमटोसेकंड एफयूसी सुविधा का व्यवस्था आरेख।

सारांश

वर्तमान लेख का उद्देश्य विकिरण और प्रकाश रसायन विभाग (आरपीसीडी), रसायन विज्ञान वर्ग (सीजी), भापअ केंद्र में विकसित अत्याधुनिक पराद्रुत प्रतिदीप्ति अप-कन्वर्जन (एफयूसी) सुविधा का एक संक्षिप्त विवरण प्रस्तुत करना और विभिन्न पराद्रुत फोटोइंड्यूस्ड प्रक्रियाओं के अंतर्गत अन्वेषण में इस सुविधा के उपयोगों पर चर्चा करना है, जो पूर्व के कम समय विभेदन वाले पारंपरिक प्रकाश भौतिकी मापों का उपयोग करके संभव नहीं हैं। इस लेख में, आरपीसीडी में विकसित एफयूसी सुविधा पर चर्चा की गई है, और बाद में इस सुविधा का उपयोग करके किए गए कुछ चयनित पराद्रुत अध्ययनों को संक्षेप में प्रस्तुत किया गया है, जिससे यह पता चलता है कि यह सुविधा इन प्रक्रियाओं के जटिल यांत्रिकी अंदरूनी पहलुओं को ज्ञात करने के लिए कैसे उपयोगी थी, जो कई व्यावहारिक अनुप्रयोगों के लिए सीधे प्रासंगिक हैं।

मुख्य शब्दः प्रकाश रसायन, पराद्रुत फोटोइंड्यूस्ड, जटिल यांत्रिकी

Ultrafast Fluorescence Up-conversion

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Fluorescence Up-Conversion Technique in Investigating Mechanistic and Kinetic Details of Ultrafast Photoinduced Processes

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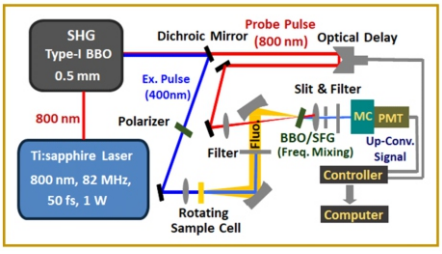

Schematic diagram of the femtosecond FUC facility developed in RPCD, BARC

ABSTRACT

Present article is aimed to provide a brief account of the state-of-the-art ultrafast fluorescence up-conversion (FUC) facility developed in the Radiation & Photochemistry Division (RPCD), Chemistry Group (CG), BARC, and to discuss the usages of this facility in exploring the insides of different ultrafast photoinduced processes, which are otherwise not possible by using conventional photophysical measurements having lower time resolutions. In this article, the FUC facility as developed in RPCD is discussed fist, and subsequently a few selected ultrafast studies carried out by using this facility have been presented in brief, bringing out how this facility was useful to get intriguing mechanistic insides of these processes, which are having direct relevance to many practical applications.

KEYWORDS: *Photochemistry, Ultrafast photoinduced processes, Intriguing mechanistic insights*

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Introduction

In Bhabha Atomic Research Centre (BARC), R&D activities in chemistry had started since the formation of this advanced research institute in 1954. In the initial years, major chemistry activities of the institute were oriented towards the development of infrastructures and facilities for advanced chemical analysis, to support different nuclear research programs of the Department of Atomic Energy (DAE). Alongside, chemistry fraternities also started perusing fundamental research to understand various radiation induced chemical processes. During mid-eighties, fundamental research in photochemical sciences was also initiated, as photoinduced processes are often complementary to radiation induced processes, and also because many photoinduced processes have direct relevance to different applied areas, namely, exotic photochemical synthesis, luminescence-based sensor development, solar energy conversion, photovoltaics applications, understanding excited state properties, and many others. Though in the initial years, photochemical studies were carried out using steady-state measurements mainly, due to the lack of time-resolved techniques, subsequently, however, different time-resolved techniques were developed in BARC for advanced photochemical studies [1-3]. In this respect, development of ultrafast photochemical techniques require to mention, and one of such technique is the femtosecond fluorescence up-conversion (FUC) facility [3]. Present article aims to discuss the important features of the state-of-the-art FUC facility developed in the RPCD, and to bring out how this facility was used fruitfully in exploring the intriguing mechanistic insides of ultrafast photoinduced processes. In the following sections, thus, we present and discuss in brief the aforementioned aspects of the FUC facility and its uses, justifying the perspective of the present article.

Fluorescence up-conversion setup: Basic aspects and instrumentation

FUC is a nonlinear frequency mixing process whereby two photon energies (ω_1 and ω_2) are mixed together to produce a sum-frequency light ($\omega_s = \omega_1 + \omega_2$) [4]. The block diagram of the femtosecond FUC facility developed in the RPCD, BARC, is shown in Figure 1. In this experimental setup, a mode-locked Ti:sapphire laser (CDP, Russia), which is optically pumped by a solid state semiconductor laser (Verdi, 5W, 532 nm; Coherent, USA) and produces ~50 fs laser pulses at a frequency of 82 MHz with central wavelength at ~800 nm and tunability range of 770-950 nm, is used as the fundamental ultrafast laser source.

In the FUC facility, the fundamental laser pulses from the Ti:sapphire laser (~800 nm) are first routed through a harmonic generator (1 mm Type-I BBO) to generate the 2nd harmonic (around 400 nm) or the 3rd harmonic (around 266 nm) light, which are used for sample excitation. A Berek compensator was used in the path of excitation light to adjust its polarization with respect to the fundamental 800 nm light which was used as gate pulse (vide infra). The excitation pulses excite the sample taken in 1 mm thick rotating quartz cell, and the emanating fluorescence from the sample is then focused onto a nonlinear mixing crystal (0.5 mm Type-I BBO) with the help of two off-axis parabolic mirrors. Meanwhile, the gate pulses are routed through a stepper motor driven optical delay line and then focused onto the same mixing crystal, overlapping spatially with the focused fluorescence light. The optic axis of the mixing crystal is tuned suitably for the best phase-matching condition to achieve efficient sum-frequency generation [4].

The sum-frequency pulses thus generated are having temporal width similar to that of the gate pulses. These pulses are separated from the residual gate pulses by using a UV band pass filter (UG 11) and focused at the slit of a double monochromator and detected eventually by using a photon counting setup (CDP, Russia). For the recording of the fluorescence decay trace of the sample, the gate pulses are delayed temporally with respect to the excitation pulses, by moving the optical delay line placed in the path of gate light. For each of the delay positions of the gate pulses, the integrated intensity (count) of the sum-frequency light is recorded for a fixed period of time, and the process is repeated with the sequential movement of the delay line, covering the whole region of the desired fluorescence trace. The plot of the counts obtained for the integrated sum-frequency light as a function of the time delay τ of the gate pulses, reproduces the

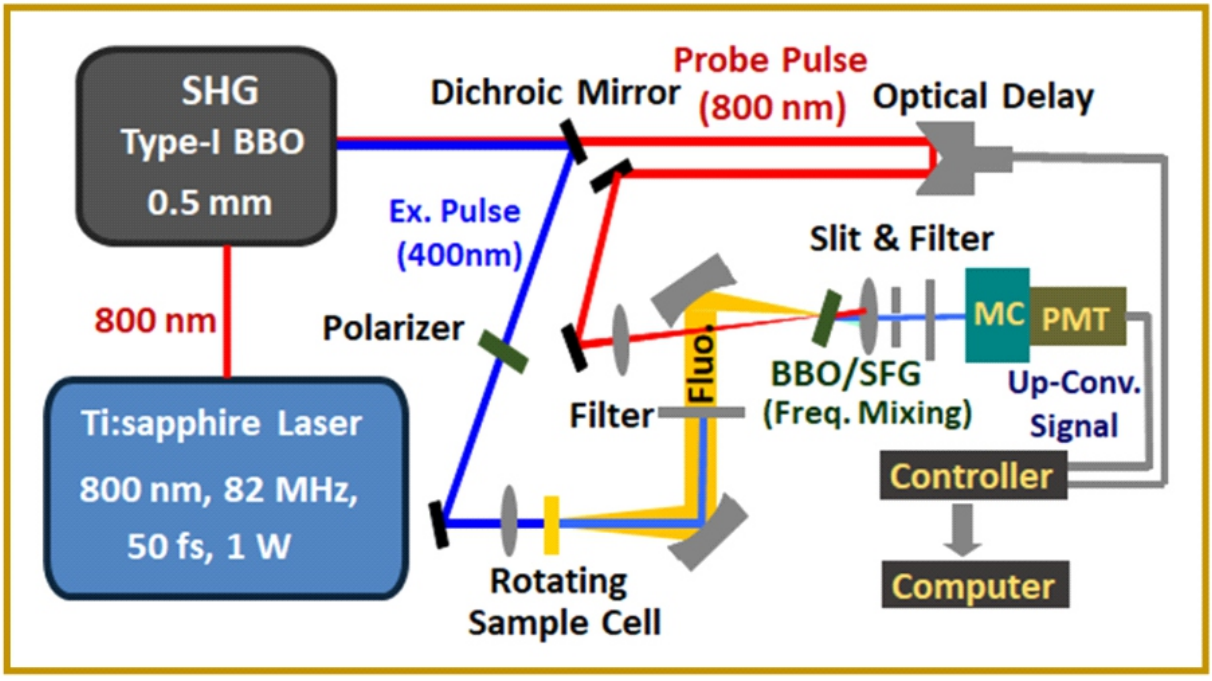

Fig.1: Schematic diagram of the femtosecond FUC facility developed in RPCD, BARC.

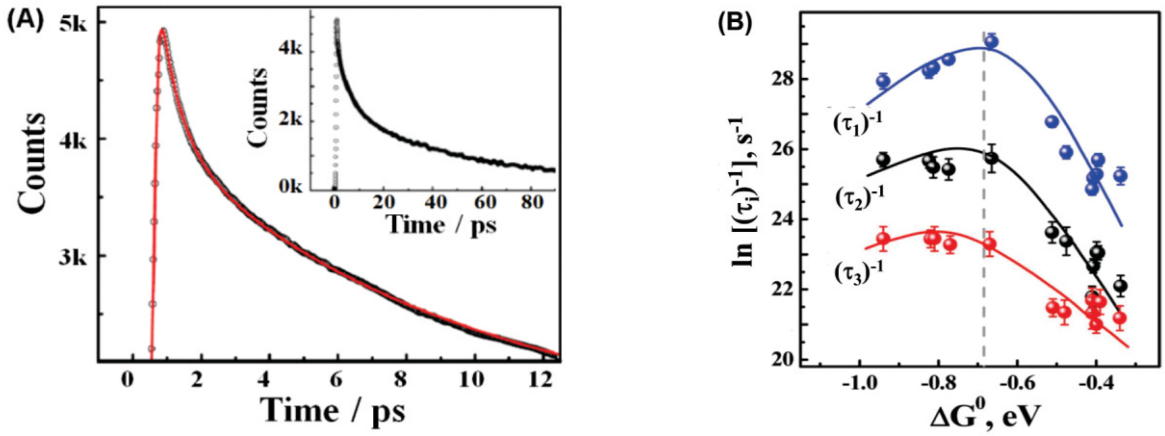

Fig.2: (A) Ultrafast fluorescence kinetic trace of donor dye coumarin 102 in cetylpyridinium chloride (CPC) micelle, where pyridinium moiety of CPC acts the electron acceptor. Initial part of the trace is zoomed in the main figure, while the full trace is shown in the inset of the figure. (B) MI behavior shown by the inverse of all the decay components (τ_1, τ_2 and τ_3) on correlating with reaction exergonicity. Figures are redrawn from reference [13].

fluorescence kinetic trace of the sample, with a time resolution similar to the temporal width of the gate pulses.

Selected ultrafast studies carried out using the present up-conversion setup

In the last about 40 years, many exciting ultrafast studies have been reported in the literature to understand the detailed insight of many chemical and physiochemical processes like, solvent relation, intramolecular relaxation, intra and intermolecular charge transfer, tortional relaxation, excited state proton transfer, photoinduced electron transfer (PET), charge recombination (CR) in ion-pairs, and so on. To be mentioned that most of these processes are directly or indirectly associated with many natural systems as well as in many practical applications. Due to space limitation, discussion on all these processes is beyond the scope of this article. Consequently, in the following we limit our discussion solely on a few of the selected ultrafast studies caried out by us using the FUC facility developed in RPCD, BARC, bringing out the intriguing results thus obtained on the concerned photoinduced ultrafast processes.

Ultrafast bimolecular photoinduced electron transfer processes

Photoinduced electron transfer (PET) is an important research topic in chemical sciences for long, especially since the development of conventional ET theory by Marcus in 1956, predicting the unique inversion behavior for the ET rates (k_{et}) on correlating with reaction exergonicity ($-\Delta G^0$), i.e. k_{et} would follow a bell-shaped curve with $-\Delta G^0$, displaying its increasing trend in the normal region ($-\Delta G^0 < \lambda$), a decreasing trend in the inverted region ($-\Delta G^0 > \lambda$), and the maximum k_{et} at the barrierless condition ($-\Delta G^0 = \lambda$), where λ is the total reorganization energy for the reaction [5]. Following this theory, enormous experimental studies were carried out, but the Marcus inversion (MI) behavior remained elusive for almost 30 years, till it was first reported by Miller and co-workers in 1984 for intramolecular charge shift reactions in radiolytically produced radical species involving donor-spacer-acceptor type of bi-functional molecules [6]. Later on, many other ET systems were found out to display MI behavior, but interestingly only for intramolecular ET and CR reactions. For bimolecular ET in conventional solvents, however, the observed reaction rates (k_{obs}) did not show the MI behavior, rather follow the Rehm-Weller behavior [7], where k_{obs} effectively saturates to the bimolecular diffusion-controlled rate (k_d) at the higher exergonicity region [5].

First report of MI behavior for bimolecular ET reaction was first reported by our group following the PET reactions in micellar systems [8-10]. In these cases, k_{obs} is found to be faster than the concerned k_d values, which are significantly lower due to constrained nature of the medium. Observed MI behavior in these cases is attributed to the non-diffusive nature of the reaction, which is understood to follow two-dimensional ET (2DET) mechanism, as proposed by Sumi and Marcus in 1986, to refine the conventional ET theory for reactions occurring under the non-equilibrium solvent relaxation condition [11]. As our initial studies were carried out using the sub-nanosecond resolved time-correlated single photon counting (TCSPC) measurements, the limited time resolution (~100 ps) of the TCSPC setup restricted us to record the ultrafast part of the kinetic traces, essential to substantiate the involvement of the 2DET mechanism. Accordingly, bimolecular PET reactions in micelles and in other constrained media were explored further, recording the ultrafast fluorescence kinetic traces of the acceptor or donor dyes in the PET reactions using FUC measurements (Fig.2 A) [12-14]. Correlating the inverse of the decay components extracted from the observed kinetic traces, we could observe clear MI behavior, not only for the fastest ET component (τ_1) but also for the other relatively slower ET components (τ_2 and τ_3) as well (Fig.2 B). These results unequivocally established that bimolecular ET in constrained media indeed progressed through 2DET mechanism than following the conventional 1DET mechanism, which is applicable only for ET reactions in low viscosity and fast relaxing solvents.

Ultrafast torsional relaxation process in molecular rotors

Molecular rotors are the fluorogenic molecules that undergo tortional relaxation in the excited state to dissipate their excitation energy in a fast non-radiative manner. The fluorescence emissions of the molecular rotors are thus extremely weak in low viscosity solvents. As the tortional relaxation are retarded very largely on increasing the solvent viscosity or on making the rotor molecules to bind with macromolecules or supramolecular assemblies, their fluorescence intensity undergoes a huge enhancement, making these dyes to be very useful fluorescence probes for various applications. Further, molecular rotors possessing target specific recognition groups can find applications in biological imaging and therapeutics. Understanding the modulations of the torsional relaxation dynamics in different microenvironments is very essential to explore the usefulness

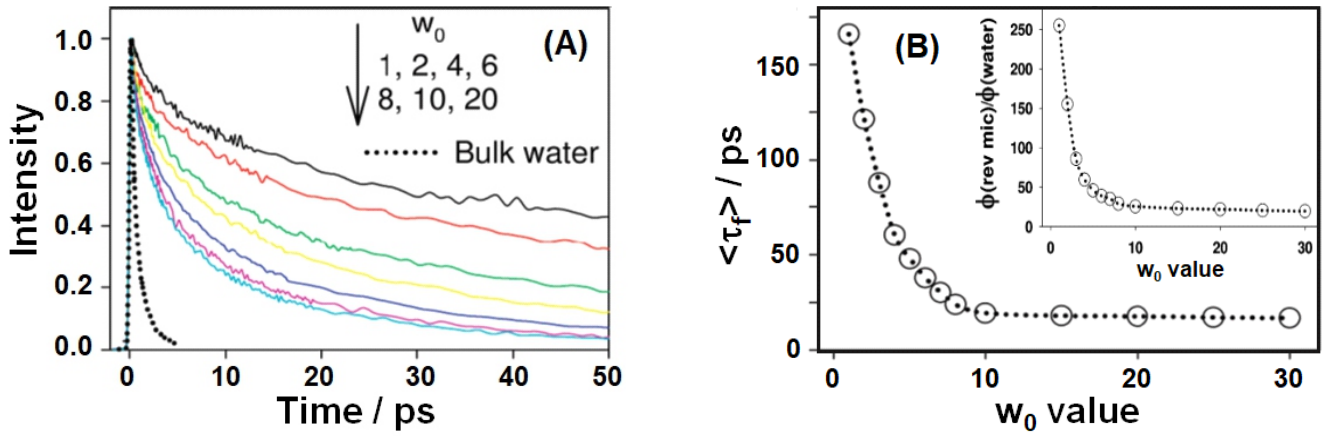

Fig.3: (A) Ultrafast fluorescence kinetic traces of ThT dye in AOT RMs with varying w_0 values. Kinetic trace for the dye in bulk water is also shown for a comparison. (B) Changes in the $<\tau_f>$ and Φ_f (inset) values of ThT in AOT RMs as a function of the w_0 values. Figures are redrawn from reference [15].

of the molecular rotors in different chemical and biological applications. In this context, good number of studies has been carried out in RPCD, BARC, following FUC measurements. Giving an account of all these studies is certainly not possible in the present article, however, in the following sub-sections we briefly present a few of these studies, highlighting the effect of supramolecular confinements and that of binding with bio-macromolecular systems on the ultrafast torsional relaxation dynamics of an important molecular rotor dye, Thioflavin-T (ThT).

Torsional relaxation of molecular rotors in confined media

In one of our studies, ultrafast deexcitation process of ThT dye was investigated in the confined water pools of aerosol-OT (AOT) reverse micelles (Rms), following FUC measurements [15]. In an earlier study, it was reported that the inverse of the fluorescence yield of ThT correlates quite linearly with the inverse of the viscosity of RMs, suggesting the usefulness of ThT for fluorescence-based micro-viscosity sensing [16]. In our study, steady-state fluorescence and ultrafast FUC measurements (Fig.3 A) were caried out to follow the changes in the fluorescence yields (Φ_f) and average fluorescence lifetimes ($<\tau_f>$) of ThT as a function of the increasing water pool size (w_0 values; water to AOT molar ratio) of the AOT RMs. Both Φ_f and $<\tau_f>$ values decreased in an asymptotic manner, approaching the limiting values in the respective cases (Fig.3 B), which are substantially higher than the corresponding values in bulk water. Observed results thus provide an intriguing inside of the confinement effect, suggesting that due to electrostatic interaction of cationic dye ThT with the negatively charged AOT head groups, and also because the water molecules in the water pools of RMs are bound to AOT head groups, the torsional relaxation of the dye in the RMs were substantially sluggish even in the cases where water pool sizes are very large, causing the torsional relaxation process in the confined environments to be quite slower than in the bulk water.

Subsequently, the w_0 depended changes in the Φ_f and $<\tau_f>$ values for ThT dye were also investigated in the cationic and neutral RMs of benzyl-hexadecyl-dimethyl ammonium chloride (BHDC) and Triton X-100 (TX-100), respectively [17,18]. In cationic RMs, the Φ_f and $<\tau_f>$ values decreased asymptotically with w_0, similar to the anionic RMs, however, the results interestingly indicated that the sluggishness introduced to the torsional relaxation is always much higher than in the anionic RMs. This observation intriguingly suggested that in RMs the principle of electrostatic interaction is largely overruled by the constitution of the nanoconfined space in the RMs [18]. Compared to cationic and anionic RMs, in neutral RMs, even though the Φ_f and $<\tau_f>$ values are much higher than in bulk water, these values and consequently the sluggishness in the torsional relaxation is quite invariant of the w_0 values [18]. This observation thus substantiated that the nature of constitution of the nanoconfined space and the localization of the probe therein determine the observed effect on the torsional relaxation of a molecular rotor in the concerned supramolecular systems.

Torsional relaxation of molecular rotors on binding with bio-macromolecules

With the perspective that the torsional relaxation of molecular rotors would be retarded significantly on binding to bio-macromolecular systems, in one of the studies, Nath and coworkers [19] used ThT as the fluorescence probe to investigate the possible structural changes of calf thymus DNA (*ct*-DNA) in the premelting temperature (T) region, i.e. $T < T_m$, the melting temperature. While primary structure of DNA changes form helix to coil form at around T_m (~75^0 C), there are also minor changes in the secondary structures of DNA in the premelting region, and these changes around the physiological temperature (~37^0 C) particularly are realized to play a very significant role in controlling many biological activities. In the literature not much studies are reported on the structural changes of DNA in the premelting region. Following steady-state and sub-nanosecond fluorescence measurements, Nath and coworkers demonstrated that both fluorescence intensity and average fluorescence lifetime of the ThT-*ct*-DNA system undergo significant changes with temperature in the premelting region, which are otherwise not observed with the standard DNA binder dyes like ethidium bromide (EB) and 4',6-diamidino-2-phenylindole (DAPI), suggesting the usefulness of ThT as a fluorescence probe to monitor the secondary structural changes of DNA as well.

Subsequent to the above study, Nath and coworkers further extended the investigation to explore the insight of the binding interaction of ThT with *ct*-DNA [20]. Ground state absorption and steady-state fluorescence of ThT showed large changes with the varying concentration of *ct*-DNA, which allowed to estimate the binding constant value for the dye-DNA system as about 3.9×10^3 M^{-1}, indicating that the dye binds very strongly with *ct*-DNA. Binding interaction in the ThT-*ct*-DNA system is perturbed very strongly by the addition of a salt, suggesting that electrostatic interaction between cationic ThT and highly negatively charged DNA plays a major role in stabilizing the dye-DNA complex. Ultrafast fluorescence kinetic

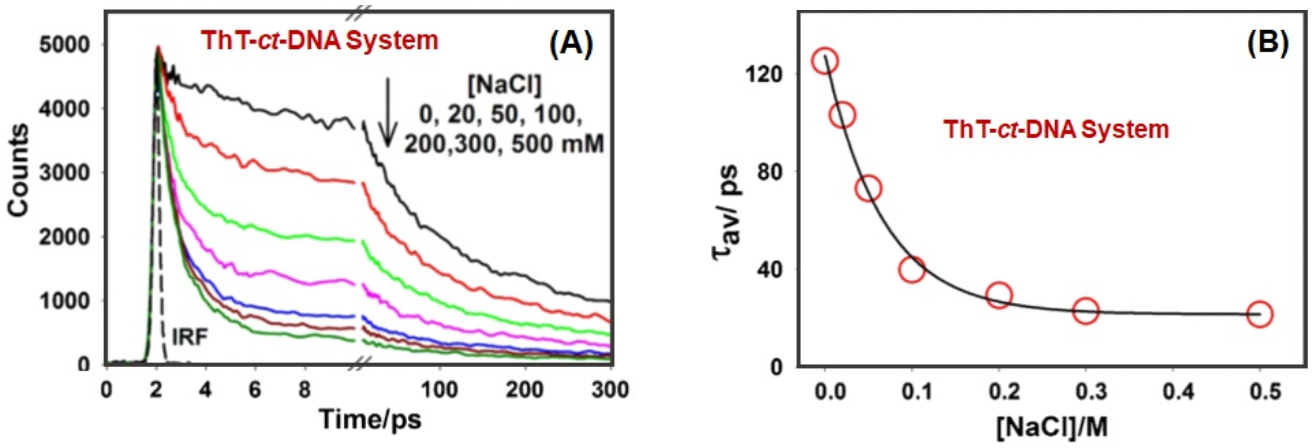

Fig.4: (A) Changes in the ultrafast fluorescence kinetic traces for ThT-ct-DNA system in the presence of varying salt concentration. (B) Changes in the <τ> values for the ThT-ct-DNA system with the changing salt concentration. Figures are redrawn from reference [20].

traces for the ThT-*ct*-DNA system as recorded through FUC measurements in the presence of varying salt concentration provided more intriguing insight of the binding interaction in this system. With the gradual addition of the salt, since the binding interaction is perturbed systematically, the ultrafast kinetic traces also concomitantly become faster (Fig.4 A) and accordingly the $<\tau_f>$ values also decrease asymptotically, approaching towards a limiting value (Fig.4 B). Interestingly, however, even in the presence of the highest salt concentration used, the limiting $<\tau_f>$ value (~20 ps) remains evidently much higher than that of the free ThT in aqueous solution (~0.61 ps). Additionally, for all the salt concentrations used, the fluorescence kinetic traces always show quite a sizeable and apparently invariant contribution of an exceptionally long (~1.4 ns) decay component. From these ultrafast results, it is evidently indicated that along with electrostatic mode of binding, a good fraction of the ThT molecules also experience the intercalative mode of binding with the DNA base pairs. Observed results also clearly indicated that while the electrostatically bound ThT molecules are slowly released from their bound state by the addition of salt, the intercalated dye molecules are hardly become free from their bound state by the influence of the salt used in the solution.

Overall, the results discussed in section 3 clearly indicate that the measurements using ultrafast FUC technique help tremendously to get intriguing insights of ultrafast photoinduced processes. Accordingly, the FUC facility developed in RPCD, BARC was used extensively to explore many other ultrafast processes, and the discussion on all these ultrafast systems is beyond the scope of the present article.

Conclusion

In the present article we provide a brief account of the ultrafast FUC facility developed in the RPCD, BARC. We also discuss the fruitful usages of this state-of-the-art facility in exploring the intriguing mechanistic insides of different ultrafast photoinduced processes studied in our group, citing and discussing the important results briefly, as obtained for some of the selected ultrafast photosystems, using the aforementioned experimental facility. In this discussion, we have tried to highlight how the ultrafast measurements helped in exploring the mechanistic and kinetic insides of the concerned photoinduced processes, which would otherwise have not been possible using conventional photochemical measurements. To be mentioned that the understanding of the mechanistic and kinetic details of ultrafast photoinduced processes are very important as these processes are directly or indirectly involved in large number of practical applications.

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